DIABETES: ITS PATHOLOGICAL PHYSIOLOGY

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DIABETES: ITS PATHOLOGICAL PHYSIOLOGY

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GENERAL EDITORS' PREFACE

THE Editors hope to issue in this series of International Medical Monographs contributions to the domain of the Medical Sciences on subjects of immediate interest, made by first-hand authorities who have been engaged in extending the confines of knowledge. Readers who seek to follow the rapid progress made in some new phase of investigation will find herein accurate information acquired from the consultation of the leading authorities of Europe and America, and illuminated by the researches and considered opinions of the authors.

Amidst the press and rush of modern research, and the multitude of papers published in many tongues, it is necessary to find men of proved merit and ripe experience, who will winnow the wheat from the chaff, and give us the present knowledge of their own subjects in a duly balanced, concise, and accurate form.

In recent years a very extensive literature has grown up, dealing with experimental researches on glycogenesis and the pathology of diabetes. In this volume the author gives the reader the gist of this literature, and the fruits of those researches which he and his co-workers have carried out, and which have led particularly to the elucidation of the control by the splanchnic nerves of the glycogenic function of the liver. Gradually facts are accumulating which throw light on the pathology of diabetes and afford us a greater power of giving rational methods of treatment, and a hope of early diagnosis and prevention of this disease.

LEONARD HILL.
WILLIAM BULLOCH.

AUTHOR'S PREFACE

THE subject-matter of this volume formed the theme of a series of eight lectures delivered during the summer session of 1912 in the Physiological Laboratory of the University of London. In preparing the lectures for publication the original plan has in general been adhered to, although here and there considerable alterations have been thought advisable.

No attempt is made to review the whole field of diabetes. On the contrary, attention has been given only to those investigations that have a more or less direct bearing on the nature of the abnormal condition or conditions in the animal body that are the cause of the disease.

It is believed by those who have had extensive clinical experience that every person who has a low tolerance towards carbohydrates is to be considered as a mild case of diabetes, even although he may be otherwise in perfect health. The condition, left untreated, may, or may not, develop into the acute disease, but it seems to be an established fact that the tendency to do so can be greatly minimized by appropriate dietary control, by which also the risk of complications is much lessened.

To apply this prophylactic treatment successfully no empirical rule for all cases can be laid down. It is necessary that each case be treated as a problem in itself, and that it be frequently examined, and the diet adjusted so as to meet its peculiar requirements. The physicians who have had most success with such cases are those who, by close attention to the results of experimental investigations, have a thorough knowledge of the physiological processes that become upset in the disease. Many of these physicians have themselves taken part in the experimental work. For those who cannot find time to do this it is hoped

that the following pages may serve as a useful review of a part of the work that has recently been done.

Since it is obviously most important that cases of incipient diabetes be diagnosed early, chapters on examination of the urine for traces of sugar, on the behaviour of sugar in the blood, and on assimilation limits, have been included in the book.

The recent work on the chemical processes involved in the production of sugar out of protein, and possibly also out of fat, that occur in severe cases of diabetes has been briefly reviewed in the concluding chapter. The subject of diabetic coma is omitted, since but little new light has been thrown on this condition by recent experimentation on animals.

To those who are primarily interested in the experimental investigations in this field which have been so numerous during recent years it should be pointed out that the researches of the author and his collaborators form the basis of the book, although an earnest endeavour has been made to incorporate the results obtained by other workers. It is hoped that no serious omissions have been made in this connection.

In conclusion, I desire to take this opportunity of thanking my collaborators, Drs. H. O. Ruh and R. G. Pearce, and Messrs. C. D. Christie and W. W. Donaldson, and my technician, Mr. E. Warnick, for their valuable assistance in the work. More especially are my thanks due to Dr. R. G. Pearce, who has been most closely associated with me, and who has, besides, kindly prepared the index. To Professor Elmer Stoll I am greatly indebted for his careful revision of the proofs.

J. J. R. MACLEOD.

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CHAPTER I

INTRODUCTORY—THE SUGAR OF NORMAL URINE

Biochemical research strives towards the attainment of a knowledge of the various chemical processes that are associated with the life of plants and animals. In order that these processes may be understood not only must the exact chemical nature of the substances that are present in each of the organs and tissues be known, but also the conditions which determine the transformation of each substance into others that are more or less related to it.

Provided with this chemical knowledge, an endeavour can then be made to find out how far similar chemical changes occur in the animal body, and in which organs and tissues and by what agencies these changes are effected. To investigate such problems, it is necessary for us, first of all, to examine the excreta for the presence of products derived from the substance in question, since from the results so obtained conclusions can often be drawn as to the general nature of the chemical change involved in the metabolism of this particular substance. We must then study the effect which is produced on the metabolism of the substance by the removal of some organ or tissue, so that we may find out what share this has in effecting the general change. We may further control the conclusions thus arrived at by investigating the changes that occur when certain organs are excised from the body and kept alive by artificially perfusing them with defibrinated blood to which the substance has been added. Such investigations constitute the subject of metabolism. The removal of the influence of certain parts of the body may be caused experimentally, or result from

disease. By comparing the metabolism of the diseased animal with that of one that is normal, a great deal about the function of the particular organ or tissue that is damaged can frequently be learned.

Among the simplest of the chemical substances which take part in the chemical processes occurring in the animal body are the carbohydrates, and a very important chapter in modern biochemical research is concerned with the changes which these bodies undergo in the animal body.

The healthy normal animal is able to oxidize completely very large amounts of carbohydrate, but this ability may become depressed, and the unused carbohydrate appear in the urine as dextrose. This condition of glycosuria, as it is called, is readily detected, and its development serves, therefore, as a valuable indicator of the state of carbohydrate metabolism. In the disease known as "diabetes mellitus," glycosuria is a most important symptom, and by observing its intensity under various conditions of diet, an immense amount of valuable information, concerning the possible sources of carbohydrate in the body, has been derived, and great progress has been made in the treatment of the disease.

In the investigation of the problems of carbohydrate metabolism, experimental and clinical investigation must therefore go hand in hand. The results obtained in the laboratory must be supplemented by observations at the bedside, not only in order that we may obtain every possible piece of information regarding the nature of the conditions which cause the disordered metabolism, but in order that we may learn how to correct the fault that exists, and therefore alleviate, if not cure, the disease. Although we cannot hope to accomplish this with any degree of certainty until we possess a clear understanding of the normal metabolism of carbohydrate, it is striking how much has already been accomplished. A few remarks bearing on this aspect of our subject, as illustrating the value of co-ordinated clinical and experimental investigation, may not be out of place.

It is now recognized that in the earlier stages of most forms of diabetes no other symptom of a deranged metabolism exists than the inability of the organism to assimilate amounts of carbohydrate which can readily be tolerated in health. So long as the diet contains less than this "assimilation limit," the urine remains sugar-free, and the person is to all intents healthy,

although we may, perhaps, more correctly say that he is a latent case of diabetes. But if there be no adjustment of the diet, and if the organism be thus persistently intoxicated by an excess of sugar, then more serious derangement of metabolism develops, and not only can less and less carbohydrate be tolerated, but the metabolism of protein becomes involved, and a portion of the protein molecule, which is ordinarily completely burnt to carbon dioxide and water, is split off as sugar, and appears in the urine. And later still, if the diabetic condition be unchecked, the damage to metabolism spreads to fats which come to be inadequately burnt, with the result that an ever-increasing portion of them is cast out in the urine as imperfectly oxidized lower fatty acids, or as acetone.

Starting merely as a carbohydrate intolerance, the disease ends with an entire disorganization of the metabolic processes concerned in the assimilation of all the proximate principles of food. Clinical studies are of great importance, if only to teach us how to check the progress of this mysterious disease; but we must go farther than this—we must try to find out the cause of the disease. For this purpose, experimental investigation is essential, for by it alone can we ascertain the exact conditions which lead to the development in lower animals of conditions similar to those found in diabetes in man. Probably the most remarkable outcome of research in this direction has been the establishment of the fact that glycosuria can be induced by a great variety of causes. In many forms of this experimental "diabetes" nothing further than a temporary glycosuria is produced, but there are other forms which closely simulate the disease itself, and which, like it, ultimately lead to the death of the animal. This division of experimental diabetes into mild and severe cases has also its analogy in the clinical field, and it is a significant fact which is now beyond dispute, that even the mildest forms of clinical glycosuria may, if not properly controlled, ultimately develop into the more serious condition, and end in death. It is therefore by analyzing the causes of experimental glycosuria, and by studying them in their relationships to one another, that we may hope ultimately to understand the exact pathogenesis of diabetes mellitus.

Yet another side of this question remains to be considered. For the metabolism of even so simple a substance as sugar in the animal organism several enzymes are necessary, and each of

these must unfold its action at a particular stage in the process ; in other words, each enzyme must prepare the molecule for the action of the next. If any one of these should fail to act, either because it is absent or because conditions are unfavourable for its activity, then the entire chemical process must go out of order, with the result that unused sugar accumulates in the organism and leads to further damage. There can be little doubt that it is because of an abnormality in the action of one of these enzymes that diabetes starts, and it becomes, therefore, a most important problem to study the nature of their activities and to compare these in the normal and diabetic animal.

The hope that some day we may be able to find out exactly which enzymic process is at fault in diabetes, and so be able, by rectifying the fault, to combat the disease, may not be so visionary as some are disposed to believe. There are, indeed, some facts which would seem to offer encouragement ; for example, judicious feeding with certain cereals, such as oatmeal, can in many cases of diabetes (and these far advanced) bring about a gradual improvement in the diabetic condition. Nor is this effect apparently due to the oatmeal starch itself, but to something else present in the grain, and therefore possibly associated with the activity of some enzyme.

The above brief sketch will serve to illustrate the general trend of modern research in the field of carbohydrate metabolism, and, although these lectures concern more particularly the results that have been obtained by laboratory investigations, we shall endeavour constantly to correlate this knowledge with the clinical, and to show, in so far as we can, how the results of physiological investigation can be made use of by the clinical investigator.

The Sugar of Normal Urine.

Although the urine of a normal animal does not usually cause reduction of copper from the cupric to the cuprous state, when Trommer's or Fehling's tests are applied to it in the usual way, it can easily be shown by more sensitive tests that it contains a certain amount of reducing substance. Sometimes, even in normal urine, the amount of this substance may become increased to such a degree that reduction occurs with the ordinary

tests, and in such cases it is not infrequently a difficult matter to decide whether or not a condition of glycosuria exists.

Apart from the necessity of bearing these facts in mind when testing the urine for the presence of sugar, it is of importance from the physiological standpoint to know in how far dextrose is responsible for the reduction, and to learn the nature of the other reducing substances. When we come to study the blood, we shall see that dextrose is present in it in simple solution, which would lead us to expect that, like other substances that are also in simple solution in blood, a certain amount of it would be present in the urine. If it is not present, or is so only to a slight extent, then we must conclude that the kidney possesses a selective power with regard to the excretion of dextrose, and, when a condition of glycosuria does become established, we must consider the possibility that it is to a derangement of the renal function, and not to an increase in the amount of sugar in the blood, that the glycosuria is due. These considerations indicate clearly the necessity of our knowing, not only whether dextrose is a constituent of normal urine, but also whether the total reducing power of this fluid bears any quantitative relationship to the concentration of dextrose in the blood.

But these problems are by no means easy ones, and there exists a great lack of uniformity in the results of different investigators. When it is simply dissolved in water, dextrose can be estimated with great accuracy by measuring the degree of reduction which a given volume of the solution brings about in a given time ; but in the case of urine, the conditions are much more complicated, for, apart from the fact that the dextrose, if really present at all, is so only in minute traces, we have constantly to guard against sources of error arising from the presence of substances, such as creatinin and uric acid. These possess reducing powers of their own, or have the property of interfering with the reduction which dextrose brings about. In the first place, it will be necessary for us to examine the total reducing power of the urine.

To measure this reducing power of normal urine, titration with Fehling's solution, as ordinarily carried out, cannot be relied upon. Even if we improve on this, and, after boiling the urine for a certain time with an excess of the reagent, collect the cuprous oxide on an asbestos filter, and ascertain its amount,

either by weighing (Pflüger-Allihn method, 1) or by titration (Bertrand method, 2*), which is the most accurate method for simple solutions of dextrose, we cannot depend on the results, because of the property which certain constituents of urine possess of dissolving cuprous oxide. In the titration methods of Pavy and Purdy, this source of error is in part surmounted by adding to the reagent either glycerine or ammonia, which completely dissolves the cuprous precipitate, and therefore makes it possible to determine, with tolerable accuracy, when all of the cupric salt has been reduced. These methods are not of sufficient accuracy for measuring the reducing power of *normal* urine, for the most serious objection to their use depends on the employment of a fixed equation for calculating how much dextrose a certain amount of reduction corresponds to.

This is inaccurate, because no allowance is made for the fact that the degree of reduction, produced by a given amount of dextrose, is considerably greater when there is an excess of cupric salt present than when there is only a small amount. Not only should there always be an excess of the alkaline cupric solution present, but specially prepared tables should be employed for calculating from the degree of reduction what amount of dextrose is present. Such tables are used in the Pflüger-Allihn and Bertrand processes.

One of the substances in urine which redissolves some of the cuprous oxide is ammonia, which is partly produced by the action of the alkali in the copper solution.

In all the above methods the copper solution contains a large amount of caustic alkali, and this, besides producing ammonia when the reagent is boiled with urine, has also the property of destroying some of the dextrose (see p. 16). To avoid these effects of the alkali, Bang (3) has introduced a titration method in which the copper solution contains sulphocyanides and carbonates, but no caustic alkali. When reduction occurs, cuprous sulphocyanide is formed, and goes into solution, the degree to which reduction has occurred being then determined by titrating with a standard solution of hydroxylamin, which reduces the unreduced cupric salt. From specially prepared tables the

* In the Bertrand process, the cuprous oxide precipitate is dissolved in a solution of ferric sulphate in sulphuric acid. During the process of solution, an amount of ferrous salt becomes produced, which is equivalent to the amount of cuprous oxide. The ferrous salt is then measured by titrating with a standardized solution of potassium permanganate. It is the most reliable method for simple solutions of sugar.

amount of dextrose can be calculated according to the volume of hydroxylamin required.

The results obtained by means of Bang's method, when applied to normal urine, are higher than those obtained by any of the other methods, but they are much more constant, and may be considered of some practical value. Thus, the average reducing power (expressed as dextrose) for persons convalescing from various diseases has been found to vary as follows : In the urines of thirty men the average was 0·238, the maximum 0·437, and the minimum 0·161 ; in the urines of twenty women the average was 0·211, the maximum 0·40, and the minimum 0·11 ; and in that of children between the ages of four and thirteen years the average was 0·196, the maximum 0·298, the minimum 0·115. In general, it was found that the specific gravity and the normal reducing power varied in the same direction (Lavesson, 4). Another observer (Andersen, 5) found the above values to vary between 0·17 in urines of ordinary colour to 0·41 in that which was highly pigmented. A urine containing above 0·5 per cent. of reducing substance (by Bang's method) must therefore be considered as unphysiological. It has been asserted that considerable dilution of the urine removes the disturbing effects of creatinin, etc ; so that, by the Bertrand process of estimation, dextrose alone produces reduction. This is, however, not the case, at least for normal urine, and the low values which the method yields are quite unreliable as an index of dextrose.

It is evident, however, that the results obtained by any of the above methods can be of very little value in helping us to follow variations in the *dextrose* content of normal urine. For this purpose it is necessary to prepare the urine in some way, so as to get rid of the non-saccharine reducing substances and of those bodies, such as creatinin, that prevent the proper precipitation of cuprous oxide. This is, however, by no means an easy matter, and it is questionable whether, even with the most elaborate methods, we can be certain of the amount of dextrose present in normal urine. Indeed, one of the most recent workers in this field has thrown doubt on there being *any* dextrose at all in this fluid (see p. 10).

There are in general two methods by which the presence of dextrose in such a mixture as urine could be recognized or its amount estimated :

1. Precipitation of the non-saccharine reducing substances by

means of various reagents, and the identification and estimation of dextrose in the filtrate by—

(*a*) Its behaviour towards yeast.

(*b*) The character of its osazone.

(*c*) The agreement between its quantitative estimation by the polariscopic and chemical methods.

2. Estimation of the reducing power of partially fractionated urine before and after allowing yeast to grow on it. The yeast destroys the dextrose, but has no effect on the other reducing substances.

Since the above methods require much time and technical skill, several tests have been evolved which react only when dextrose is present in pathological amount in the urine, and which are therefore of great value for the diagnosis of mild cases of glycosuria.

It would be out of place to describe here in detail each of these methods—such information can be obtained by consulting the various original papers or the practical textbooks—but it is important for us to consider briefly the principles upon which they depend and some of the results that have been obtained by their use.

1. *Removal of the Disturbing Substances from the Urine.*—If we desired to estimate the amount of dextrose or other sugar in a very dilute solution, we should, of course, as the first step, concentrate this by evaporation in faintly acid reaction.* But in the case of the urine, mere evaporation to small bulk (in acid reaction) does not render the detection of dextrose any more certain than in the original urine. To detect the dextrose, it is necessary to remove from the urine not only the substances which themselves cause reduction, but also, as far as possible, those which interfere with the reducing action of dextrose itself. There is, however, great uncertainty and difficulty in separating such substances from urine without at the same time running the risk of removing or destroying dextrose, or causing it to be produced out of other substances by action of the reagents. These difficulties are partly due to our comparative ignorance of the exact chemical nature of the disturbing substances. So far, we know that they include creatinin, uric acid, and urochrome (6). Thus, 7 parts of creatinin have the same reducing power as 4·8 parts of dextrose, and 10 parts of uric acid as

* Even the smallest trace of alkalinity destroys dextrose.

3·47 parts of dextrose, the degree of reduction being—unlike that of dextrose—directly proportional to the amount of substance present (7). In urine containing average amounts of creatinin and uric acid, it has been found that 26·3 per cent. of the total reducing power is due to creatinin, and 7·8 per cent. to uric acid, thus leaving about 66 per cent., or two-thirds, of the total reducing substance unaccounted for. It is said that a large part of this unknown fraction is due to urinary pigment (8) (urochrome), which is in conformity with the well-known fact that highly coloured urines have a high reducing power. The remainder possibly represents carbohydrates, of which, besides dextrose, there may be some that do not ferment with yeast, such as isomaltose and lower dextrines (9).

The methods that have been employed for the purpose of separating these substances include precipitation with mercuric nitrate (Schöndorff, 10), lead acetate, or phosphotungstic acid, and absorption by means of charcoal.

Mercuric nitrate precipitates from urine many substances, including creatinin, uric acid, pigments, and inorganic salts. After the removal of the mercury precipitate by filtration, and of the excess of mercury in the filtrate (by sulphuretted hydrogen or sodium hydroxide and zinc dust), it is possible to evaporate the urine to small bulk without causing destruction of the sugar, provided, of course, that the reaction during evaporation is kept faintly acid. Although the resulting solution gives a more or less typical reduction with Fehling's solution, it is necessary, before attempting to estimate the amount of reducing power, to get rid of the considerable quantities of salts that have been produced by interaction of the reagents employed. This can be done by precipitating with excess of alcohol and using a watery solution of the evaporated alcoholic extract. Unfortunately, however, this treatment with alcohol is quite likely to cause a destruction of some of the dextrose (Oppler, 13).

There is a lack of uniformity in the results that have been obtained by the investigators who have employed this method. Schöndorff, for example, found 0·0274 per cent., and Bang (11) and his co-workers 0·08 per cent. of dextrose. It is probable that the higher results obtained by the latter group of workers are due to their having employed zinc dust and sodium hydroxide for removing the excess of mercury and the Bang method for estimating the reducing power of the final filtrate (*cf.* p. 7).

Pavy (12) precipitated many of the interfering substances by means of acetate of lead, and after removing this precipitate by filtration, found that the addition of ammonia to the filtrate caused a second precipitate which contained the dextrose. By suspending this precipitate in water and treating it with sulphuretted hydrogen, lead sulphide separated out, and the dextrose went into solution, and could be recognized, after filtration and evaporation, by reduction, by yeast fermentation and by the microscopical appearance of the osazone crystals. Pavy states that the residual reducing power equals about 0·05 per cent. dextrose.*

An exhaustive and critical investigation of this subject has recently been published by Oppler (13). In the method employed by this worker urine is treated with phosphotungstic acid till no more precipitate occurs, allowed to stand in the dark, filtered, the filtrate shaken with acetate of lead, and again filtered free from lead tungstate. Excess of lead is removed by sulphuretted hydrogen, and after suitable evaporation the extract can be used for (1) fermentation by yeast; (2) estimation of the reducing power by Bertrand's method; (3) polariscope examination. For estimation of the dextrose the last-mentioned method is especially recommended by Oppler, and when Bertrand's method is used for this purpose, the addition to the mixture of reagent and sugar solution of 40 per cent. ordinary (96 per cent.) alcohol is advocated in order to keep the boiling-point of the mixture about 85° C. No doubt the manner of heating—*e.g.*, the time taken to reach the boiling-point—is of importance in Bertrand's method.†

In several of the urines thus examined no trace of dextrose could be found, and this fact has led Oppler to doubt the commonly accepted conclusion that dextrose is a constituent of normal urine; in others, there was only from 0·001 to 0·01 per cent. On the other hand, yeast-destructible reducing substance to the extent of 0·04 per cent. was often found present.

It would take us beyond the scope of these lectures to enter further into a description of the complicated chemical procedures that have to be employed in order to determine whether

* Acetate of lead has also been employed by Andersen (*loc. cit.*), but was not found to remove so much of the non-saccharine substances as mercuric nitrate.

† We have found that when the solution is brought to the boil in two minutes, and kept boiling for three minutes, the most accurate results, as calculated from Bertrand's table, are obtained. *Cf.* also Peters (*loc. cit.*).

or not dextrose is really a constituent of normal urine. There are many sources of error—for example, the preparation of typical dextrosazone crystals, by means of phenyl-hydrazine, is insufficient proof of the presence of dextrose, because a secondary formation of this sugar (out of glucosamin, for example), may occur ; nor is the fermentation method entirely reliable, because of the fact that optically active substances may be produced by the growth of yeast (see also p. 35). As the matter stands at present, we must conclude that there is no unassailable evidence that dextrose is a constituent of normal urine.

On the other hand, there are many conditions bordering on the pathological in which dextrose does appear in small amount in the urine, so that it is necessary for us to have at our disposal some quantitative method by which such mild forms of glycosuria can be detected and measured.

The methods described above, even if they were reliable, are too complicated and time-consuming to render them of use for this purpose. We must content ourselves with one by which a constant proportion of the reducing substance is removed, and which yields us with a fraction of the urine still containing, besides all the dextrose, a more or less constant amount of non-saccharine reducing substance. The total reducing power of this fraction will, of course, be dependent to a much greater degree upon any dextrose it may contain than is the case with unfractionated urine, and, by observing it from day to day, we should be able—at least approximately—to follow changes in the dextrose content. For this purpose we must endeavour to remove all of the colouring matter and as much as possible of such substances as creatinin and uric acid. This can best be accomplished by shaking the urine for five minutes with 10 per cent. blood charcoal (Merck) in the presence of 25 per cent. glacial acetic acid, or 15 per cent. chemically pure acetone (Rona and Michaelis, 14).* By this treatment the charcoal absorbs all the colouring matter, about 80 per cent. of the uric acid, and about 70 per cent. of the creatinin, and since the colouring matter is also removed, we obtain a filtrate with which colorimetric titration, as by Bang's method, can be very accurately carried out.

Charcoal by itself absorbs some dextrose, but it does not do

* The original statement by Bang and Bohmansson, that 5 per cent. hydrochloric acid also prevents absorption of dextrose, is far from correct (15).

this in the presence of acetic acid or acetone in the above proportions, as is evident from the following table :

PREVENTION OF ABSORPTION OF DEXTROSE BY TEN PER CENT. BLOOD CHARCOAL.

Percentage of Dextrose before adding Charcoal, etc.	Substance added along with the Charcoal.	Percentage of Dextrose after adding Charcoal, etc.	Method used for estimating Dextrose.
Hydrochloric acid—			
1. 0·510	25 per cent. concentrated HCl.	0·320	Bang
2. 0·512	25 Do.	0·280	,,
Acetone—			
3. 0·512	10 per cent. chemically pure acetone	0·510	,,
4. 1·012	10 Do.	0·960	,,
5. 1·012	25 per cent. acetone	1·012	,,
6. 3·190	Charcoal alone	1·710	
,, ...	2 per cent. acetone	2·470	
,, ...	4 Do.	2·850	Polariscope
,, ...	12 Do.	3·220	
3·370	15 Do.	3·390	
Acetic acid—			
7. 1·020	25 per cent. acetic acid	1·050	Bang
8. 0·950	25 Do.	0·944	Bertrand
9. 0·202	25 Do.	0·207	Bang
10. 0·202	15 Do.	0·186	,,

It must, of course, be remembered that acetone itself may cause slight reduction, and in such a case a correction must be made for the amount of acetone used. But it is possible to obtain acetone that shows no reduction. Acetic acid in the above proportion is perfectly reliable. It is important always to employ the purest blood charcoal (Merck), for many varieties of charcoal absorb sugar even when acetone, etc., are present. The clear liquid which is obtained after filtration can be employed either for polariscopic examination or for titration by Bang's process. The gravimetric method, by Pflüger-Allihn, is not so satisfactory, for the cuprous oxide does not separate properly, and a dark and gummy mass collects on the filter. Bertrand's process is satisfactory with some urines, but not so with others.

Donaldson and Christie, using the above method, estimated the amount in grammes of reducing substance excreted in the twenty-four hours' urine over a period of several days, with the following results :

C. 1·341 ; 1·595 ; 1·03 (a cathartic was taken, and the volume of urine was very small) ; 1·592 ; 1·394 ; 1·414 ; 1·449 ; 1·270 ; 1·200 ; D. 1,848 ; 1·014 ; 1·010 ; 1·06 ; 1·38 ; 1·185 ; 1·258. It is of interest to note that the daily variations are not great, and that C. excreted on an average distinctly more reducing substance than D.

Both men were in perfect health, doing a moderate amount of muscular work, and living on a mixed diet containing a fairly constant amount of carbohydrate. No relationship could be made out between the amount of carbohydrate in the food and the amount of reducing substance excreted. It was intended to study the influence of a gradual increase in the carbohydrate ingestion on these values, but pressure of other work compelled the abandonment of the investigation.

2. *Ascertaining the Diminution of Reducing Power produced by allowing Yeast to grow in the Urine.*—We have already referred to this method as applied to urine that has been treated with precipitating reagents. Even in such cases, as has also been pointed out, care must be taken in concluding that the diminution in reducing power produced by the fermentation represents dextrose, and, in any case, such elaborate procedures as these methods involve would be too time-consuming for routine work.

We have also seen that after-treatment of the urine with charcoal, in the presence of acetone or acetic acid, a great proportion of the non-saccharine reducing bodies are removed, but not the dextrose. In order to determine this alone, and so to eliminate entirely the error involved by those reducing bodies that are still present in the filtrate, it would at first sight appear simple enough to measure the diminution of reducing substance produced by incubating some of the urine with yeast. By such a method it has been computed that about 17·8 per cent. of the total reducing substance in normal human urine is due to dextrose—that is to say, that about 0·05 per cent. of this sugar is present (16). But the method is untrustworthy, as will be evident from the following observations : Faintly acid urine was incubated for twenty-four hours with thoroughly washed and pressed fresh brewer's yeast,* and after filtering off the yeast and clarifying with charcoal, etc., it was titrated by Bang's method. Another portion of the same urine was directly

* Baker's yeast—*e.g.*, Fleischmann's—is unsuitable because it contains starch (tapioca).

clarified with charcoal, etc., and similarly titrated. Instead of finding that fermentation caused some reducing substance to disappear, however, it was sometimes found that the reducing power of the fermented preparation was *greater* than that of the unfermented (17). Such results as the following were obtained :

Reducing Power Per Cent. (Bang).

Before Fermentation.	After Fermentation.
0·083	0·072
0·039	0·042
0·087	0·062

Prolonging the fermentation to forty-eight hours did not give any more satisfactory results, and we were led to suspect that yeast, by its growth, must produce substances that are capable of partially reducing Bang's solution (*cf.* also 17A). We have sufficient evidence to hand to show that this is the case—at least, for certain forms of yeast. Although this fact renders the method quite inapplicable as an exact one for the estimation of dextrose, it has, nevertheless, been extensively employed for this purpose, particularly in the case of blood (see p. 33). It therefore becomes of importance for us to learn the conditions under which these substances are produced. With this object in view, we have determined the reducing power of incubated suspensions of yeast in water and in dextrose solutions, not only by Bang's method, but also by those of Pflüger-Allihn and Bertrand, with the following results : A filtered 10 per cent. suspension of yeast in water after twenty-four hours' incubation, gave 0·07 per cent. reduction (expressed as dextrose), and after forty-eight hours, 0·08 per cent. The same solutions did not produce any measurable reduction when investigated by Bertrand's method.

A similar suspension of yeast in 1 per cent. dextrose gave, after twenty-four hours' incubation, 0·07 per cent. by Bang's method, and 0·008 per cent. by the Allihn-Pflüger method.

Occasionally, however, a slightly higher reduction than that indicated above was obtained with Bertrand's method, but never anything like that obtained by Bang's. When the yeast suspensions were examined immediately after mixing, only the smallest trace of reduction occurred, even with Bang's method ; it became more marked as incubation proceeded. It is not as yet known of what nature the reducing substances produced by yeast may be.

Treatment of the fermented solution with acetic acid and charcoal seems to remove a certain part of the reducing substance, but, of course, this does not render the method usable.

The evident impossibility of estimating the amount of dextrose by the difference in reducing power before and after allowing yeast to grow on the solution—at least, when Bang's method is used for titration—prompted us to see whether the same principle could be employed for estimating dextrose by the use of Bertrand's method. At the outset we may state that we are convinced that Bertrand's method for sugar estimation is in many particulars superior to that of Bang. The end-point of the titration is sharper (for no colorimetric titration exceeds in accuracy that in which permanganate solutions are used), the solutions are very easily standardized and easy to prepare (which is by no means the case with Bang's solutions), they keep indefinitely, and the reagents employed are considerably cheaper. But, as already mentioned, when we attempt to employ the Bertrand method with urine, even when this has been clarified by means of charcoal, the cuprous oxide precipitate, besides being impure, does not as a rule settle well, so that it cannot be collected on the asbestos filter. It must, further, be pointed out that to employ Bertrand's method in the case of normal urine, 40 c.c. of urine must be used and a proportionate amount of the reagents. This is necessary because of the low reducing power of normal urine, but it interferes with the accuracy because the mixture takes a longer time to boil than that for which the tables have been prepared.*

The general conclusion which we may draw is that the quantitative methods for estimating the reducing power of urine are not of sufficient accuracy for the detection of slight degrees of glycosuria, and this is true, even when we remove a part of the interfering substances. It becomes necessary for us to employ qualitative tests—that is to say, tests which react positive only when there is more than a certain amount of dextrose in the urine.

Qualitative Tests for the Detection of Slight Degrees of Glycosuria.—There is, of course, no difficulty in recognizing a large excess of dextrose in the urine, for the specific gravity of the urine, the production of measurable quantities of carbon dioxide

* The supplementary tables for small amounts of dextrose, prepared by Moeckel and Frank (*Zeitsch. f. Physiol. Chem.*, 1910, lxv., p. 323), must be used.

gas on fermentation with the yeast, the ready reduction of alkaline copper or bismuth solutions, the influence on the plane of polarized light, etc., are eminently satisfactory tests in such cases. But it is a difficult matter to decide in doubtful cases whether or not a condition of glycosuria exists. And it is most important that we should be able to do this, for, if there really is an excess of dextrose, however small, it indicates that something is amiss with the utilization of carbohydrates in the organism ; it is a danger signal, which, if heeded and the proper treatment applied, may unquestionably enable us to stave off the incidence of what might afterwards prove a deadly diabetes.

Until the quantitative methods for dextrose estimation in normal urine have been more thoroughly worked out, we must depend on more or less arbitrary tests. There are three such tests that can be depended upon. They are named after their authors, the " Worm-Müller," " Nylander-Hammarsten," and the " Benedict " tests.

By these tests the creatinin, uric acid and other non-saccharine reducing substances in urine do not bring about a typical reduction, nor does the small amount of dextrose which is said to be normally present. A slight increase in dextrose, however, causes a typical reduction. On account of the importance of knowing how to apply the tests properly, we shall describe them in some detail, and at the same time discuss their respective reliability. Before doing so, however, it is important to bear in mind that in all three of them it is not the sugar itself which brings about the reduction, but a decomposition product, which is temporarily formed as a result of the heating of the sugar with alkali.

But this decomposition product is not stable, and the rate with which it becomes changed into a substance, which can no longer reduce, is proportional to the temperature of the solution and the concentration and nature of the alkali present. It is because of the rapid destruction of this intermediary reducing substance that no reduction is visible when a trace of dextrose is boiled with undiluted Fehling's solution, but becomes so when the mixture is allowed to cool somewhat.

In urine, also, it must be remembered that there are substances, chief of which is probably creatinin (Maclean, *loc. cit.*), which inhibit the reducing action of the newly formed substance, and so allow the caustic alkali present in Fehling's solution to

destroy it before any reduction has occurred. Thus, although it is possible by Fehling's test to detect 0·001 per cent. of dextrose in water, there must be at least 0·1 per cent. of dextrose in urine to give a positive reaction with this reagent.

The Worm-Müller reagent * contains less caustic alkali than that of Fehling, and in performing the test the urine is not boiled with the reagent, but the two are mixed together at a temperature of about 90° C. and then allowed to cool off. The test is performed in the following way :

Five c.c. of urine is boiled in one test-tube, and 3·5 c.c. of a mixture of 2·5 c.c. alkaline Rochelle salt solution and 1 c.c. copper solution in another. After allowing the test-tubes to stand for twenty-five to thirty seconds, the blue reagent is poured into the urine, when, if any pathological amount of sugar is present, a finely suspended yellow precipitate develops within ten minutes. Mere change of colour of the solution without the formation of precipitate does not indicate a positive result. When such a change of colour occurs without the formation of a precipitate, the test must be repeated with a higher proportion (2 to 4 c.c.) of the copper solution. A precipitate of phosphates may sometimes also cause confusion. This precipitate, however, is quite different from that of the finely suspended precipitate of cuprous oxide, for it soon separates in small masses. If there is still doubt as to the presence of reduction, the test-tube should be set aside for twenty-four hours, after which time, if reduction due to an excess of dextrose has occurred, a red deposit of cuprous oxide will have gathered at the bottom of the tube.

When a positive reaction is obtained, it is advisable to ferment some faintly acidified urine with 10 per cent. yeast for twenty-four hours, and after removal of the yeast by filtration, to apply the test in exactly the same manner as before. It will now be negative in cases of glycosuria.

It is unquestioned that this is a most valuable test in doubtful cases, but it is not infallible. On the one hand, it can happen that a urine with no abnormal amounts of dextrose, but an excess of other reducing substances, may give it ; and, on the other, although an excessive amount of dextrose be present, the resulting cuprous oxide may fail of precipitation.

* The following reagents are used : Solution I., 2·5 per cent. $CuSO_4$ in water. Solution II., 10 grammes Rochelle salt made to 100 c.c. with 4 per cent. NaOH solution.

The test is empirical; it rests on the assumption that the reducing substances present in the normal urine—*i.e.*, the urochrome, creatinin, uric acid, and dextrose—will produce no more cuprous oxide than can be held in solution by the various urinary constituents, such as creatinin and ammonia, which possess the property of dissolving it, but that any excess of dextrose will produce more cuprous oxide than can be thus dissolved. Two possible sources of fallacy therefore exist: First, that there may be an insufficient amount of the substances which dissolve cuprous oxide, so that this becomes precipitated, even although there is no excess of it produced ; and, secondly, that there may be an excess of these dissolving substances, so that an abnormally strong reduction occurs without any cuprous oxide being precipitated.

Recognizing these possible sources of error, it is therefore important to see in how far actual experience with the test has proved its worth. Pflüger (18) and Schöndorff (19) have used the test extensively. In an investigation of the urines of 144 patients in the surgical and gynæcological wards of the Bonn Clinic, collected shortly after operations, the test was invariably negative. Schöndorff tested the urines of 334 soldiers resident in the Bonn garrison, and unexpectedly obtained positive results in all but 18 of the cases (5·4 per cent.). In those urines in which it was positive, the reaction varied considerably in intensity, being feeble in 180, moderate in 107, and very marked in 29. In this last group the test was as marked as it is found to be in the urine of untreated cases of diabetes mellitus. The cause of these curious results was furnished by an investigation of the diet; it being found that the men were consuming excessive quantities of starch (over 600 grammes of garrison bread daily), as a consequence of which *glycosuria e amylo* had become established (see p. 210). These unexpected findings prompted Schöndorff to examine by the same test the urine of students and other persons living on an ordinary diet. The results were quite different: out of 53 cases, a positive reaction was obtained in 8 (15·1 per cent.), and in these only feebly.

Of the other investigators who have criticized the Worm-Müller test, the conclusions of Hammarsten (20) and Bohmansson (21) are of importance.

Bohmansson examined twenty urines of fever and cardiac patients, and applied the Worm-Müller test, after clarifying the

urine by means of acid and charcoal. In 45 per cent. of these the test was negative, but in the others distinct reduction occurred (without, however, the deposition of Cu_2O insisted on by Pflüger, except in three cases), even although quantitative estimation by the method described above (p. 11) did not show that abnormally large amounts of dextrose were present. It was also observed that two urines, containing an excessive amount of dextrose—*i.e.*, more than 0·05 per cent.—failed to give the reaction. Worm-Müller (22) himself places the delicacy of his method at 0·025 of dextrose over and above the normal amount. He determined this by adding varying quantities of dextrose to urines that had been fermented with yeast so as to rid them of dextrose. By adding known amounts of dextrose to normal unfermented urine, we have obtained a positive reaction with 0·06 per cent., but negative with 0·03 per cent.

Another test is that of Nylander-Hammarsten. It is a modification of an old test (Böttger-Alem), and depends on the reduction of bismuth oxide to metallic bismuth in the presence of caustic alkali.* Since prolonged heating causes reduction to occur, even with normal urine, it is most important in performing this test to abide strictly by the directions. In our experience the test is most satisfactorily applied in the following manner (23):

Ten c.c. of *protein-free* urine is mixed in a test-tube with 1 c.c. of the reagent. A white precipitate of phosphates is produced. The test-tube is then placed for exactly five minutes in a boiling-water bath. On removal, the phosphate precipitate is more marked, and this, as well as the supernatant fluid, has turned grey or black in cases where an abnormal amount of dextrose is present. A faint brownish discoloration of the solution alone is not considered a positive result; the precipitate must also show a definite metallic greyness.

Regarding the delicacy of the test, a 0·08 per cent. watery solution of dextrose gives a positive reaction. The presence of even small amounts of protein interferes with the delicacy of the test by holding the reduced bismuth in colloidal suspension. Protein may further cause confusion because of darkening due

* The reagent is made by dissolving 4 grammes Rochelle salt in 100 c.c of an 8 per cent. NaOH solution (by weight). This solution is heated on the water-bath, and 2 grammes bismuth subnitrate dissolved in it. Filter if necessary.

to the formation of sulphide of bismuth, on account of sulphur split off by the action of the caustic alkali.

The addition to urine of even large amounts of uric acid, urates, or creatinin, does not give a positive result when the test is applied in the proper manner. Some observers assert, however, that it sometimes reacts positive in urine, even after this has been fermented by yeast (Bohmansson, Lavesson). In none of these cases, however, was the test applied as directed above. By studying the results of this test applied to urines in which the actual percentage of dextrose was measured by the difference in reduction produced by yeast, Lavesson found it positive in four urines which did not contain more than 0·05 per cent. dextrose. He found it negative in a urine containing 0·065 per cent. We have found the test to react positive with normal urine, to which 0·03 per cent. of dextrose had been added ; it was negative with 0·015 per cent. We prefer it to the Worm-Müller test.

The third test (Benedict's, 24), unlike the others, has only recently been introduced, and although, on this account, it has not been sufficiently examined and criticized to permit us to consider it as an infallible test, yet it seems to be very valuable and reliable. It has, moreover, the great advantage of extreme simplicity. The reagent consists of a solution of sodium carbonate and citrate containing cupric sulphate.* About 5 c.c. of the reagent are mixed with about 10 drops of urine and the mixture boiled for two minutes. In the presence of more than 0·3 per cent. of dextrose a yellowish precipitate, equally distributed throughout the solution, separates, or, if somewhat less than this percentage of dextrose be present—but still a pathological amount—the precipitate may separate only after partial cooling. The colour of the precipitate is unimportant ; hence the test can be accurately applied in artificial light. Sometimes a greyish precipitate of urates may appear, but this is a source of confusion only until some practice has been had with the test. In such cases, another test (Nylander's) should be applied as a control.

* Dissolve 17·3 grammes cupric sulphate in about 100 c.c. water ; also dissolve 173 grammes sodium or potassium citrate, and 100 grammes sodium carbonate in about 700 c.c. water. Slowly pour the copper solution, with constant agitation, into the alkaline citrate solution, then cool and make up to 1,000 c.c. The reagent keeps indefinitely in ordinary bottles, for the citrate does not, like Rochelle salt, undergo spontaneous change into reducing substances.

We have seen how it is really not dextrose itself, but a decomposition product produced by the action of alkali on it, that causes the reduction when Fehling's solution is used. In the presence of alkaline carbonates, as in Benedict's test, this reducing substance is more gradually produced, and is only slowly destroyed, so that it has time, as it were, to bring about reduction of the copper.

This reagent does not become reduced with creatinin, uric acid, or chloroform; indeed, the only substances sometimes occurring in urine which can reduce it, besides dextrose, are homogenistic and glycuronic acids. It is an easy matter, when either of these is present, to distinguish it from dextrose by fermenting the urine with yeast, for after the fermentation the reduction, if due to dextrose, will disappear.

By adding known quantities of dextrose to normal urine, we have found, on two minutes' boiling, that the test reacts positive when 0·03 per cent. is added (assuming that normal urine contains 0·5 per cent. of dextrose, this would indicate that about 0·08 per cent. of dextrose can be detected). After the addition of 0·015 per cent. of dextrose, a negative result was always obtained. It may be added that when the time of boiling is two minutes the reduction precipitate usually forms during the boiling.

Before concluding, it may be well to point out that it should be made a practice to apply at least two tests in every doubtful case of glycosuria. We believe that the Nylander and Benedict tests are the most serviceable for ordinary work. The more difficult Worm-Müller test should be applied in very doubtful cases, but it requires more experience and care than the others. In every case in which a doubtful reaction is obtained with any of these tests it should be made a practice to ferment some of the (faintly acidified) urine for twenty-four hours with yeast, and then to filter and re-apply the test.

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CHAPTER II

THE SUGAR OF THE BLOOD

That the blood may contain a sugar-like substance was first of all shown in 1775 by Dobson in a case of diabetes, but it was not until seventy years later that the presence of a similar substance, as a constituent of normal blood, was established by Claude Bernard, who also discovered that the amount of this sugar became increased when rabbits were rendered glycosuric by puncture of the floor of the fourth ventricle. This observation showed clearly that, in certain forms of glycosuria, the reducing power of the urine reflects more or less accurately its intensity in the blood. That the presence of reducing substance in excess in the urine does not, however, invariably depend on an increase in the reducing power of the blood was established in 1886 by v. Mering and Minkowski, who found that the administration of phloridzin to animals caused an increase in the urinary reducing power, accompanied either by no change, or by a diminution in the reducing power of the blood. Other facts, pointing in the same direction, were gradually accumulating. One of these had, indeed, been recognized by Claude Bernard, for he noted that the urine of a healthy animal contains but the merest trace of reducing substance as compared with that present in the blood. So far as the tests available could reveal, there did not appear, in cases of experimental glycosuria, any increase in the reducing substance in the urine until a considerable excess of it had accumulated in the blood.

During the last two or three years a great revival of interest in the reducing substances of the blood has taken place, and it is with the recent work which this revival has prompted rather than the older work that we will deal in this chapter. There are several aspects of the question that must be considered. These concern—

1. The total amount of reducing substance in the blood.
2. The distribution of the reducing substance between corpuscles and plasma.
3. The chemical nature of the reducing substance.
4. The distribution of the reducing substance when sugar is added to the blood.
5. The possibility of the existence in blood of loose combinations between the reducing substance and some of its other constituents, and the possible existence in blood of substances which, although they do not themselves reduce, can yet be readily converted into reducing substances.
6. The relationship between the reducing substance of blood and that of the urine.
7. Glycolysis.

Before we proceed with a discussion of these questions it may be well to pause for a moment and consider in what way their solution will be of value in understanding the mechanism of glycosuria. The simplest conception of the cause of glycosuria is that it is due to hyperglycæmia—that is to say, to an increase in the sugar content of the blood.* Were all of the sugar present in the blood in a state of simple solution in the plasma, there would be no reason for doubting this simple explanation. On the other hand, should the red corpuscles contain some sugar, and their envelopes be partially impermeable to this substance, then it is evident that there might be a considerable change in the amount of sugar in the *whole* blood without any change occurring in that which is in simple solution in the plasma ; or there might be a considerable increase in the sugar in the plasma which would fail to produce any change in that found in the whole blood if there were a concomitant deficiency in the corpuscles. Even if it should be shown that the red corpuscles contain no sugar—on account of their envelopes being completely impermeable to it—we should yet have to consider whether the sugar in the plasma exists therein in simple solution, or whether some of it may not be in some form of combination with other constituents of the blood, for, obviously, that sugar only which is in simple solution in the plasma will be available to the tissues or excretable by the urine.

* For brevity's sake, we will hereafter usually designate the reducing substance as *sugar*. It must be remembered, however, that this may not be strictly accurate (see p. 33).

The conditions under which sugar is contained in the blood may, in other words, be compared with those which apply in the case of carbon dioxide in blood. Only a small part of this gas is in simple solution in the plasma, the greater proportion being in chemical combination, as carbonates or bicarbonates, in the plasma, or combined in some way in the corpuscles. And just as we speak of the tension of carbon dioxide as meaning that percentage of this gas which is in simple solution in the plasma, and of total amount as meaning all the carbon dioxide which can be extracted from the blood, in whatever way it may be contained therein, so with sugar we may have to deal with a tension as well as with a total amount.

It is believed that the average tension of carbon dioxide in the blood is more or less constant, although the amount may vary considerably, there being a sort of equilibrium between the gas in simple solution and the total acid radicles in the blood, and so with sugar it may be that the tension is constant although considerable variations occur in the total amount.

On the other hand, if all of the sugar should exist in simple solution and be readily diffusible through the envelopes of the erythrocytes, then amount and tension would be the same, and the general problem would be considerably simplified.

The Total Reducing Power of the Blood.

This is measured in the usual way, by finding how much cupric salt a given quantity of blood can change to the cuprous state. But before mixing the blood and cupric solution it is necessary to remove the proteins from the former, and herein lies the source of all the difficulty and uncertainty of blood-sugar estimations; for, by the ordinary methods of protein precipitation, it is almost impossible to remove the protein without at the same time carrying down some sugar. The protein precipitates are copious and more or less gelatinous, which makes it very difficult to wash them free of adherent sugar. The original protein precipitant used by Claude Bernard was sodium sulphate; other workers used alcohol, or mercuric nitrate, or chloride (*cf.* 2), but in all cases in which the reagents were used difficulty was experienced in washing the precipitates so as to be certain that all sugar had been removed from them.

To circumvent this difficulty Schenck (1), assuming that the sugar is equally distributed between the precipitate and fluid, advised using an aliquot portion of the filtrate, for the actual sugar estimations ; but the results of his method do not compare well with those obtained by the use of methods in which the precipitate is completely extracted with water. A great improvement in the technique was applied by Waymouth Reid (3), who used phosphotungstic acid as the protein precipitant, the advantage of this reagent being that the precipitate which it produces is of such a consistency that it can be thoroughly extracted of all traces of sugar by grinding in a mortar with cold water. We have had considerable experience with Reid's method, and have tested it as to its reliability in every possible way. It is undoubtedly very accurate, provided care be taken thoroughly to extract the precipitate, and to keep the filtrates, during their evaporation, faintly acid in reaction. But it is costly and time-consuming, and requires great care in carrying out.

Since, until recently, these two methods have been the most extensively used, it is of importance for us to see how far the results obtained by them are in accordance with one another. When we compare the amounts of total sugar in different portions of the same blood, as estimated by the methods of Schenck and Reid, there is always found to be more by the latter than by the former method (4). The following table clearly shows this :

Reducing Substance in 100 Grammes Blood by—

Reid's Method.	Schenck's Method.	Percentage of Difference.
0·151	0·129	14
0·165	0·135	18
0·231	0·207	10
0·143	0·129	10

It is evident that mercuric chloride, which is used in Schenck's method, precipitates some reducing substances which are not precipitated by phosphotungstic acid, as used in Reid's method. When comparative observations are to be made on a series of bloods, it is therefore essential that the same method for removal of protein be employed throughout, although, even then, we do

not know but that there may be considerable error entailed on account of irregular precipitation of certain of the non-saccharine reducing substances. To minimize such an error, it is of course important to use the method which removes least of these. Until recently the method best conforming with this requirement has been that of Waymouth Reid.

The results obtained by the use of the older methods, although evidently subject to error, are, however, of considerable value and interest.

Some of the most important of them are given in the table on p. 28.

Certain general conclusions can be drawn from the results recorded in the table. The most important of these are—

1. The average amount of sugar in the arterial blood of dogs, shortly after being etherized, is 0·15 to 0·17 per cent. when Reid's method is employed. Occasionally the amount may be considerably above or below this average, but in most cases it is quite near it.

2. The venous blood of unanæsthetized dogs contains only about 0·1 per cent. of sugar, according to the results obtained by Schenck's method. The differences between these two groups of observations may be accounted for, first, by the difference in the methods of estimation employed; secondly, by the effect of ether, which causes a slight degree of hyperglycæmia in dogs ordinarily fed (see p. 187). It is possible also that there may be a smaller amount of sugar in venous as compared with arterial blood (see, however, p. 48).

3. The considerable variations in the sugar content of healthy human blood are partly to be explained on account of improper analytical methods. But even with the same method it is evident that considerable variations exist between the blood of different individuals (Liefmann and Stern).

4. In the rabbit, the cat, and the dog, the amount of sugar increases somewhat as a result of boiling the protein-free extract of blood with acid. By this treatment it would seem that sugar is liberated from some form of combination.

A great improvement over older methods has recently been introduced by Rona and Michaelis (5), who have shown that the proteins of blood, but none of the sugar, can be precipitated by

shaking the laked and diluted blood with colloidal ferric hydroxide (dialysed iron), and then adding a small amount of an electrolyte, such as sodium sulphate.

Since it is important, for the accurate application of the

REDUCING POWER OF NORMAL BLOOD RENDERED PROTEIN-FREE BY THE OLDER METHODS.

Animal.	Percentage of Reducing Power (as Dextrose).	Observer.	Method used—Remarks.
Dog	0·1 to 0·25	Bernard (*cf.* Liefmann and Stern, *Bio. Zeitschr.* 1906, vol. i., p. 301)	—
,,	0·17	Vosburg and Richards (*Amer. Journ. Phys.*, 1903, vol. ix., p. 38)	Waymouth Reid method; sixteen results (etherized dogs)
,,	0·16	Underhill (*Journ. Biol. Ch.*, 1905, vol. i., p. 113)	Waymouth Reid method (etherized dogs)
,,	0·15 0·103 Min. 0·207 Max.	Macleod (*Amer. Journ. Phys.*, 1907, vol. xix., p. 388; 1908, vol. xxii., p. 373)	Waymouth Reid method; thirty results (etherized dogs)
,,	0·098	Embden, Lüthje, and Liefmann (*Beit. z. ch. P. u. P.*, 1907, vol. x., p. 265)	Venous blood of unanæsthetized dogs (Schenck method)
Man	0·17	Frerichs, v. Mering, v. Noorden, Naunyn (quoted from Liefmann and Stern, *Bio. Zeitschr.*, 1906, vol. i., p. 299)	—
,,	0·12 to 0·33		
,,	0·1 ,, 0·15		
,,	0·05 ,, 0·15		
,,	0·08 ,, 0·09		
,,	0·086	Liefmann and Stern (*loc. cit.*)	Schenck method; venous blood (twenty results)
,,	0·105 Max.		
,,	0·065 Min.		
Rabbit	0·1089 (before hydrolysis)	Pavy ("Carbo. Metab.," 1906, p. 13)	Killed by pithing; heart-blood; twelve results; alcohol precipitation of proteins
,,	0·1495 (after hydrolysis)	Do.	Do.
Cat	0·088 (before hydrolysis)	Do.	Do.
,,	0·140 (after hydrolysis)	Do.	Do.
Dog	0·086 (before hydrolysis)	Do.	Do.
,,	0·130 (after hydrolysis)	Do.	Do.

method, that we understand the principle upon which it depends, it may not be out of place to outline briefly this principle here. Colloids are charged with positive or negative electricity. When two colloids of opposite electrical sign are mixed, so that there is exact neutralization of the electrical charge of the one by that

of the other, precipitation of both will occur; but if there be a slight excess of either colloid in the mixture, the precipitation will be incomplete. On the other hand, if one colloid be present in great excess of the other, the adsorption compound will acquire many of the physico-chemical properties of the colloid that is in excess; it will, for example, become precipitated when the excess colloid is precipitated. Colloids are precipitated when they are mixed with small amounts of electrolytes, the cause of the precipitation being the neutralization of the electrical charge of the colloid by the charge of opposite sign carried by the electrolyte—that is to say, a positive colloid is precipitated when its positive charge of electricity becomes neutralized by the negative electricity carried by the anion of the electrolyte. Thus positive colloids such as ferric hydroxide are more readily precipitated by such electrolytes as sulphates or phosphates than by chlorides.

Applying these principles to blood, we find that proteins are amphoteric—*i.e.*, may carry charges of electricity of either sign—so that they can form adsorption compounds with both negative and positive colloids. Thus they combine with kaolin, which is a negative colloid, as well as with dialysed iron, which is positive. After mixing blood-serum or laked blood with an excess of either of these colloids, the protein-colloid compound thereby formed acquires the properties of the added colloid, and becomes precipitated along with the added colloid when a suitable electrolyte is added.

Thus, when colloidal iron has been used, precipitation occurs on adding a small amount of a sulphate, because the negative electricity of the sulphion neutralizes the positive electricity of the colloidal iron.

The reducing substances of blood are not precipitated during the above process, so that after the removal, by filtration, of the precipitated proteins, an aliquot portion of the filtrate can be evaporated to suitable volume, and used for the estimation of sugar by the reduction or polariscopic methods. Furthermore, since the added reagent is precipitated at the same time as the protein, no necessity exists, as in the older methods, for subsequently removing the excess of reagent from the filtrate. For these two reasons, the method is very much more rapid than any of the older ones. It is, besides, more accurate and much less expensive.

SUGAR OF NORMAL BLOOD RENDERED PROTEIN-FREE BY ADSORPTION METHOD.

Animal.	Percentage of Reducing or Dextro-rotating Substances (as Dextrose).	Remarks as to Method, Observer, etc.
Rabbit	0·100	Polariscope used. When results controlled by Bang titration, they were found to be higher by 25 to 30 per cent. Blood removed from ear vein of rabbits and jugular vein of dogs. Animals unanæsthetized. Four animals in each group (Oppler and Rona, 6).
,, ..	Max. 1·119	
,, ..	Min. 0·061	
Dog ..	0·085	
,, ..	Max. 0·102	
,,	Min. 0·073	
Dog ..	0·157	Average of ten estimations on arterial blood of anæsthetized animals.
,, ..	Max. 0·210	
,, ..	Min. 0·115	
Cat ..	0.280	Average of four estimations.
,, ..	Max. 0·355	
,, ..	Min. 0·154	
Rabbit	0·118	Average of four estimations.
,,	Max. 0·150	
,,	Min. 0·102	
Man ..	0·075	One estimation.
		In the majority of the above cases the polariscopic method was used ; in others Bertrand's method (Rona and Takahashi, 7).
Man ..	0·080	Average of ten individuals. Unclotted (fluoride) blood employed. Polariscopic method (Rona and Döblin, 8).
,, ..	Max. 0·128	
,, ..	Min. 0·048	
Dog ..	0·174	Blood from vena cava opposite hepatic veins in anæsthetized dogs. Bang's titration method (Macleod and Pearce, 9).
,, ..	Max. 0·219	
,, ..	Min. 0·073	

Although all of these estimations were made on blood from which the proteins had been removed by means of colloidal iron, there is seen to be a considerable variation in the results. This variation exists not only when we take the results of different observers, but is also evident in those of the same observer. In the case of dog's blood the differences are undoubtedly partly due to the fact that in one series of observations venous blood of unanæsthetized dogs was employed, whereas in the other it was the arterial blood from lightly narcotized animals. The method employed for estimating the dextrose in the protein-free extracts also makes a difference, the results being higher with reduction methods than with the polariscope. This is especially the case when Bang's method is chosen. Although the polariscope is very suitable for most purposes, it must be remembered that there are some errors involved in its use (see also p. 35).

Even after we allow for all sources of irregularity in the results,

a considerable variation is evident, not only in the blood-sugar of animals of different species, but also in that of animals of the same species. On this account it is never trustworthy to compare the sugar content of one animal with that of another for the purpose of deciding whether a change in the amount has occurred, as a consequence of some experimental condition. Whenever possible, the comparison should be made with the amount found in the same animal under normal conditions; and when this cannot be done, as in clinical investigations, no decision as to whether a larger or a smaller amount than usual is present should be arrived at, unless a great many results are taken into consideration.

The Distribution of the Sugar between the Corpuscles and Plasma.

Disregarding for the present the exact chemical nature of the substances to which blood-extracts owe their reducing or optically active properties, we will proceed to inquire into the distribution of these substances between corpuscles and plasma. There are, in general, two methods by which this may be determined:

1. Oxalate or defibrinated blood is centrifuged, and the percentage reducing or polarizing power of plasma and whole blood separately measured. In another sample of blood the relative volumes of corpuscles and plasma are determined by means of the hæmatocrit.* From these data the absolute amounts of reducing substances in plasma and corpuscles may be calculated, as shown in the following example: The plasma contained 0·175 per cent. reducing substance, the whole blood 0·128 per cent., and the hæmatocrit gave the relative volumes of plasma and corpuscles as 62 and 38. Therefore, the 62 c.c. plasma contained 0·108 gramme, and the 38 c.c. corpuscles 0·128 − 0·108 = 0·020 gramme, or 0·0526 per cent. The results are best stated in percentage amounts, since we are then able to compare the relative sugar concentrations. In the table of results this method is designated as the *indirect*.

2. The corpuscles may be washed free of adherent serum, etc., by centrifugalization, then laked, the hæmoglobin precipi-

* For approximate work the proportion of corpuscles may be taken as 40 per cent. in dog blood and 50 per cent. in human blood.

tated by means of colloidal iron, and the reducing or polarizing power of the evaporated filtrate and of the plasma separately determined. Although this is, theoretically, the more *direct* method, it is not entirely a satisfactory one, because the prolonged washing of the corpuscles which is necessary to rid them of serum is apt to cause the loss from them of some of the sugar. The general nature of the results obtained by different observers is illustrated in the following table :

PERCENTAGE REDUCING OR DEXTROROTATORY POWER (EXPRESSED AS DEXTROSE) IN—

Whole Blood.	Red Corpuscles.	Plasma.	Animal.	Remarks.
(1) 0·161	0·107	0·180	Dog.	Average of 8 dogs. *Direct* method. Polariscope. Amount in whole blood agreed with added amounts in corpuscles and plasma in five cases. Not so in three cases. (Rona and Michaelis, 10).
(2) 0·098	0·042	0·137	,,	*Direct* method. Reduction. (Lepine and Boulud, 11).
(3) 0·163	0·140	0·178	,,	Average of seven dogs. *Indirect* method. Polariscope and Bertrand, which gave same results (Rona and Takahashi, 12).
(4) 0·108	0·020	0·154	Rabbit	Same methods as in preceding (Rona and Takahashi, 13).
(5) —	0·07 to 0·08	0·270	,,	*Direct* method. Bang reduction (Lyttkens and Sandgren, 14).
(6) —	0·054	0·122	Ox	*Direct* method. Bang's reduction (Lyttkens and Sandgren, 15).
	0·077	0·134	Horse	
	0·048	0·128	Pig	
	0·055	0·304	Cat	
	0·073	0·280	Guinea-pig	
(7) 0·104	—	0·093	Man	*Indirect* method. Schenck reduction. No precautions against glycolysis (Hollinger, 16).
(8) 0·07 to 0·09	—	0·08 to 0·11 (max. 0·12 min. 0·07)	,,	*Indirect* method (Frank, 17).
(9) —	0·069	0·103	Man (placenta blood)	Direct method. Bang reduction. (Lyttkens and Sandgren, 18).

Practically all of the investigations agree in showing that there is less sugar in the corpuscles than in an equal volume of

plasma. The only dissent from this conclusion is by Edie and Spence (19), who concluded that the corpuscles do not contain any sugar.

But the partition is not constant, for the blood of different individuals may show considerable variations in this regard. Thus, in samples of blood from thirty patients, suffering from various diseases, Frank (17) found the whole blood to contain a trace more sugar than the plasma in four, practically the same amount in five, and distinctly less in the whole blood than in the plasma in twenty-one.

Not infrequently there is a discrepancy between the amount of sugar found in the whole blood and that computed by adding together the sugar separately determined in the corpuscles and plasma (10). This may be due to the setting free of "actual" from "virtual" sugar during the manipulations, for it is more marked when the blood is heated with acid (20) (see p. 43). In certain forms of hyperglycæmia the difference is very marked; thus in one specimen of rabbit blood the total reduction was 0·17 per cent., whereas the added reduction was 0·217 per cent; in another these values were 0·345 and 0·407 respectively.

The Nature of the Sugar in Blood.

So far, for convenience sake, in quoting results we have expressed the total reducing or dextrorotatory substance in the blood as if it were entirely due to dextrose. We must now proceed to ascertain whether this is really the case, or whether the blood, like the urine, may not contain other reducing and dextrorotatory bodies besides dextrose. In order to determine the actual amount of dextrose present, the method has been to subject the protein-free blood-extract to the action of yeast, when the resulting diminution in the reducing or dextrorotatory power has been considered as an accurate measure of the dextrose content. By applying the methods described in the previous section, it is possible to estimate the fermentable sugar for corpuscles and plasma respectively.

The yeast, which should be fresh and washed brewer's yeast,* is mixed with the faintly acidified protein-free and evaporated filtrate, and incubated for twenty-four hours. It is of advantage

* Baker's yeast, as obtained in the market, is mixed with starch, and should not be employed for this work.

to employ sodium phosphate for precipitating the colloid, since the presence of this salt in the final filtrate favours the fermentation process. Simple as this method would appear to be, there is by no means uniformity of opinion among the various workers who have used it as to the proportion of the total sugar of blood that is due to dextrose. Before attempting to explain the cause for this disagreement, we shall give some of the results in tabular form :

THE PERCENTAGE OF FERMENTABLE AND NON-FERMENTABLE SUGAR IN BLOOD AS DETERMINED BY—

*A. The Polariscope (and Indirect Estimation of the Corpuscular Moiety).**

Animal.	Blood.		Plasma.		Red Corpuscles (calculated).
	Before Fermentation.	After Fermentation.	Before Fermentation.	After Fermentation.	
Dog ..	0·180	0·001	0·178	0·002	0·144
Cat ..	0·377	0·000 or minus	0·321	0·001	0·194
Rabbit ..	0·108	0·006	0·154	0·000	0·002 (?)

B. Bang's Titration Method (and Direct Estimation of the Corpuscular Moiety).†

Animal.	Blood (added from Plasma and Corpuscles).		Plasma.		Corpuscles.	
	Before Fermentation.	After Fermentation.	Before Fermentation.	After Fermentation.	Before Fermentation.	After Fermentation.
Man (placenta and venous blood) ..	0·172	0·100	0·103	0·040	0·069	0·060
Rabbit ..	0·277	0·055	0·270	0·048	0·070 to 0·080	0·070 to 0·080
Ox ..	0·176	0·089	0·122	0·036	0·054	0·053
Horse ..	0·211	0·113	0·134	0·036	0·077	0·077
Pig ..	0·176	0·090	0·128	0·046	0·048	0·044
Cat ..	0·359	0·089	0·304	0·035	0·055	0·054
Guinea-pig ..	0·353	0·103	0·280	0·032	0·073	0·071

There is a striking dissimilarity between the results of the two groups of observers. Those who employed the polariscope found that after fermentation all of the optically active substance had been destroyed, indicating therefore that dextrose alone is

* Rona and Takahashi, 21. † Lyttkens and Sandgren, 22.

present both in the corpuscles and plasma. Those who employed Bang's titration method found, on the other hand, that a considerable amount of reducing substance remained after the fermentation, and that this was very nearly the same in amount in plasma and corpuscles. Such a recurring want of uniformity in the results suggests, of course, some fundamental error in one or other of the methods employed, and, as we have already seen in our first lecture, this must depend on the fact that there are substances produced by the growth of yeast which are capable of causing some of Bang's copper solution to become reduced. It is interesting to note that the residual reduction found by Lyttken and Sandgren for the corpuscles, as well as for the plasma, is pretty much the same as that which we have found after twenty-four hours' incubation of yeast, either in water or in dilute sugar solutions. It is evident that the results of Lyttkens and Sandgren are due to error, and cannot be accepted.

Since the non-saccharine reducing substances produced by yeast do not bring about precipitation of cuprous oxide from Fehling's solution, they are not included in estimations for dextrose when Pflüger-Allihn's or Bertrand's method is used.

Although the results obtained by the use of the polariscope are therefore very much more reliable than those obtained by the use of Bang's method, it is important to bear in mind that some sources of error exist for them also. The chief of these are as follows :

1. The readings are subject to an experimental error, which, with such small amounts of optically active substance as the solutions employed contain, may make the total possible error very large (18).

2. No allowance is made for lævorotatory substances present in the blood. That such exist can be clearly seen from certain of the above results.

3. Yeast by its growth may add substances to the solution which have rotatory powers of their own.

By other methods of chemical analysis, it is said, the glycuronates, pentoses and jecorins, can be identified in normal blood (23). These substances are capable of reducing, but they do not ferment with yeast; they represent the non-saccharine reducing substances spoken of above. No doubt glycuronates and pentoses may, under certain conditions, be present in blood in quite considerable amounts, but it is a question whether they can contribute in any appreciable degree to the reducing power

of normal blood. Jecorin does not always contain a dextrose group (to which it owes its reducing qualities), but even when this is the case, as in dog's blood, the degree of reduction, as compared with the total reducing power of blood, is very small (from 2·5 to 5 per cent. of the total reduction). It must be remembered, also, that this substance is formed artificially when an alcoholic solution of lecithin is evaporated to small bulk in the presence of dextrose (Bing, 24).

We may, I think, conclude with safety that there is no certain evidence of the existence of other than yeast-fermentable sugars in normal blood. These include, besides dextrose, maltose and cane-sugar ; for yeast contains *invertases* that hydrolyze these disaccharides to dextrose and lævulose.

For the identification of these fermentable sugars it is necessary to prepare osazone crystals from the protein-free extracts of blood. When phenyl-hydrazine is used for this purpose in the usual way, dextrosazone is readily produced (25), and it is said that maltasozone may also be formed. An osazone is also obtained when methyl-phenyl-hydrazine is employed, which indicates that lævulose must be present, since it is with this monosaccharide alone that methyl-phenyl-hydrazine reacts (Neuberg and Strauss, 26). This conclusion conforms with those of earlier observers, who, however, employed less trustworthy tests (Lepine, 27). Lævulose has also been detected, by the use of the above reagent, in ascitic and pleuritic fluids, its amount in these being increased when large quantities of it are present in the food. Its presence in blood may, in part, account for the lower values obtained for total sugar by polariscopic, as compared with reduction methods of estimation. Glycuronates will also have this effect.*

The Distribution of the Sugar in Blood during Hyperglycæmia.

As has already been pointed out at the beginning of this lecture, it is the sugar present in solution in the plasma rather than that in the corpuscles which is of importance in connection with sugar exchange between the tissues and the blood, and with the sugar excretion by the kidney. It must therefore be of great interest to know how the distribution of sugar in the blood

* Glycuronic acid rotates the plane of polarized light to the right, but its salts, the glycuronates, rotate it to the left.

(*i.e.* between plasma and corpuscles) behaves when more sugar is added to it—that is to say, when a condition of hyperglycæmia exists. Such studies have been made in three ways: (1) By adding sugar to the drawn blood; (2) by overfeeding an animal with sugar, or by producing hyperglycæmia in some other way; (3) by examining the blood of diabetic patients.

1. In the earlier observations on the permeability of the envelopes of washed red blood-corpuscles towards sugar in drawn blood, it appeared as if no sugar could penetrate (28), but more recent work has shown that in the case of unwashed corpuscles penetration does occur (29). The experiments upon which these conclusions depend were performed by adding varying amounts of a concentrated dextrose solution to fresh human blood, kept from clotting by sodium fluoride. The actual quantities of dextrose added varied between 0·3 and 0·5 per cent. In a minute or two after the addition of the sugar a portion of the blood was centrifuged, and the dextrose content determined, in plasma and whole blood, by the Rona and Michaelis method. By comparing the results so obtained with those obtained in a sample of the same blood before the sugar was added, penetration of the corpuscles by the dextrose could easily be shown.

The following is a typical result:

Per Cent. Sugar in—

	Blood.	Plasma.	Red Corpuscles.
Before adding sugar ..	0·094	0·098	0·089
After ,, ,, ..	0·375	0·401	0·322

The penetration was, however, insufficient, in the time of the experiments, to bring about equalization of sugar in corpuscles and plasma, and it is stated that even after prolonged standing (twenty-four hours) this does not occur. When the sugar is added to defibrinated blood, its penetration of the red corpuscles is less marked.*

It is significant that, although washing destroys the permeability of the corpuscular envelope for sugar, it does not

* There is evidently a great difference in the penetrability of the envelope of the red corpuscles, according to whether they are washed or unwashed. The significance of this fact in connection with hæmolysis experiments need scarcely be pointed out.

cause the sugar originally present in the corpuscles to be washed out. This conclusion depends on the observation that the dextrose content of serum alone is the same as that of a mixture of the serum and of the saline that has been used to wash the corpuscles (30). Had sugar been washed out, the latter results should have been higher than those for serum alone.

2. When the sugar is added to undrawn blood—that is to say, when a condition of hyperglycæmia becomes established in the body—the increase in sugar seems also to affect both corpuscles and plasma ; but the results of different investigators do not agree as to the extent to which this penetration of the corpuscles occurs. The effects of hæmorrhage, of injection of an excess of sugar, and of administration of adrenalin, have been more especially studied in this connection. As a result of hæmorrhage in the rabbit, Lyttkens and Sandgren (31) found only a slight increase in the reducing power of the corpuscles (from 0·07 to 0·14 per cent.), and they thought that this was due entirely to non-saccharine reducing substances ; in the serum, on the other hand, the increase was found to be due entirely to fermentable sugar (from 0·22 to 0·54 per cent.). Similar results were obtained when the blood in adrenalin hyperglycæmia was examined.

On the other hand, Rona and his co-workers found, when hyperglycæmia was produced in the dog by means of hæmorrhage, or by the excessive ingestion of dextrose, that the plasma and corpuscles participated in the increase of dextrose to about an equal degree (32). These discrepancies are, no doubt, to be accounted for by differences in technique (see p. 13).

3. The partition of sugar between the corpuscles and plasma has been investigated in cases of *pathological hyperglycæmia*, and in other diseases by Bönninger (33), Hollinger (34), and Frank (35). Bönninger states that in a case of so-called "renal" diabetes the increase in sugar was confined largely to the plasma. Hollinger, as a result of investigations on the bloods of thirteen patients, all exhibiting hyperglycæmia, came to the opposite conclusion, for he found that in the majority—viz., nine—there was more sugar in the whole blood than in the serum, thus indicating that the corpuscles contained more than the plasma.*

* Frank believes that a possible error was incurred in these observations on account of glycolysis (*cf.* 35).

Frank examined the blood in fourteen similar cases, and found that the increased amount of sugar usually affected the plasma to a greater extent than the corpuscles. In four of them, however, the whole blood gave higher results than the plasma. Only certain of the patients were diabetic; others were suffering from pneumonia, hepatic cirrhosis, etc. The actual results obtained in the diabetic cases were as follows:

Reducing Power of Whole Blood Per Cent.	Reducing Power of Plasma Per Cent.
0·296	0·320
0·200	0·230
*0·095	0·140
0·100	0·137
0·150	0·280
0·202	0·257

Michaelis and Rona determined the distribution of dextro-rotatory substance in the blood of three cases of diabetes mellitus, and found it to vary, not only in the blood of different patients, but also in the blood of the same patient examined at different times. The variations were, however, not marked, and the conclusion drawn is that the corpuscles and plasma participate to about an equal extent in the increase.

A very important practical point presents itself as a result of these conclusions—viz., should we take the whole blood or the plasma for the purpose of assaying the reducing power? The importance of the question becomes evident when we consider that an increase in the reducing substance in the one or other constituent of blood might occur, along with a decrease in the other, so that the whole blood would show no change.

After all, however, as has already been set forth, it is the plasma-sugar which is of importance, both in connection with tissue metabolism and with excretion of sugar into the urine. The sugar in the corpuscles is usually distinctly less in amount than that in the plasma, and it is to be considered as a possible reserve of sugar, from which the plasma, if necessary, may replenish its supply. For these reasons, then, it seems clear that the results obtained from plasma are more to be depended upon for deciding whether hyperglycæmia or hypoglycæmia exists than those obtained from whole blood.

* Latent case after treatment.

Is the Sugar of the Blood Free or Combined?

"Free" and "combined" are terms that are in common use to express the possible conditions of sugar in the blood, and there are all sorts of views as to the manner of combination of the latter portion. The existence of such compounds would, of course, render much of the plasma sugar incapable of ready diffusion through animal membranes, so that a thorough examination of this question is of great importance.

According to Lepine and Pavy, even the sugar which is obtained after precipitation of the proteins, by alcohol or sodium sulphate, is believed to have existed in the living blood in a combined state, and to have become dissociated from the substance with which it was combined by the chemical manipulations necessary for its estimation. Besides this readily dissociated sugar, these authors, however, believe that there also exist firmer compounds of sugar in the blood. In one group of these the combined sugar is liberated by boiling with weak acid, or by the action of some ferment. In another group a still firmer combination exists—namely, one in which the sugar is intimately incorporated with the protein molecule, and dislodged from it only after tearing apart the molecule by the use of strong reagents.

But we must decide whether *any* part of the dextrose in blood exists in a combined state before the question as to the possible nature of such a compound need be discussed. The evidence in favour of the view that sugar is combined in the blood is very largely of a theoretical nature. Pavy (37) assumed the presence of such a compound because he did not see how otherwise the sugar in the blood could escape being excreted in the urine. "When circulating through the kidney," so he writes, "it [dextrose] cannot be prevented from escaping like other small molecular bodies, and making its appearance in the urine." Lepine (38) comes to the same conclusion, because he found that, by dialysis of blood-plasma, obtained by centrifuging cooled blood against an ice-cold isotonic (0·75 per cent.) saline solution for two hours, none of the dextrose in the plasma had diffused into the saline.

When the dialysis is allowed to proceed for four or five hours, however, some dextrose does diffuse (Edie and Spence, 39), so that

experiments of this type are quite inconclusive. In order to have the fluid on the two sides of the dialyser membrane identical in every respect, except with regard to the dextrose, Asher and Rosenfeld (40) dialysed fresh blood, kept from clotting by means of sodium fluoride, against blood that had been fermented with yeast until all of its dextrose had disappeared, and also containing fluoride. It was found, after dialysis had proceeded for some time, that no dextrose remained in the blood in the dialyser, thus indicating that it had diffused through the parchment and been destroyed by the yeast. They concluded that the sugar must exist in a free state in the blood, as otherwise it would not have diffused. This experiment is, however, also unconvincing, for although it is true that the disappearance of the dextrose in the blood contained in the dialyser must have been due to its diffusion into the mixture of blood and yeast—for no yeast could have penetrated the dialyser—yet this sugar may have existed in other than a free state ; some of it may have been combined, but subsequently broken down by invertin—a soluble ferment secreted by yeast and capable of passing through parchment. Invertin can dissociate sugar from glucosides and similar compounds (Pflüger, 41).

But even without such an action by invertin, there is a serious objection to all experiments of the above type in the present connection on account of the fact that *any* compound of sugar might quite readily be broken down as a result of the removal of free sugar by dialysis, for, even supposing that there were both free and combined sugar present, they would stand in a certain equilibrium to one another, so that a lowering of the amount of free sugar, because of its diffusion into the saline, would cause a liberation of sugar from the compound, and the dialysis of this would proceed until all had disappeared.

These few observations will indicate the enormous difficulties which face us in attempting a solution of this most important question. These difficulties are occasioned by the fact that the combination of sugar, if it exists at all, must be of the loosest possible nature, so loose that even the feeblest of the chemical reagents commonly employed for precipitating proteins can dissociate it. We must employ some reagent which will precipitate protein without causing any chemical compound of it with sugar to be disturbed. Such reagents have been

discovered by Rona and Michaelis (42) in certain colloids such as kaolin and colloidal iron, which, as already explained, form adsorption compounds with protein, but not with sugar (see p. 27).

If, as is supposed, dextrose exists in blood as a colloidal compound with protein, then it should be adsorbed by one or other of the above colloids, for one carries a negative, the other a positive, electrical charge. But comparison of the amount of dextrose in the filtrates obtained with kaolin and colloidal iron does not show any difference. The results of the colloidal methods also correspond with those of the older methods. Such results could scarcely be obtained did there exist even the feeblest kind of combination between sugar and protein.

Further evidence of the same type is furnished by the observation that whereas charcoal adsorbs both protein and dextrose from a solution containing the two substances alone, yet it adsorbs the protein, but none of the dextrose, when acetone is also present in the solution (see p. 12). The acetone cannot cause any compound of protein and sugar to be dissociated, but, being more adsorbable by charcoal than dextrose, it prevents this from being adsorbed. We have already studied the application of this principle in connection with the removal from the urine of reducing substances other than dextrose.

The conclusion arrived at, that all of the dextrose exists in simple solution in the blood, does not, however, depend solely on these observations. Corroborative evidence is furnished by the following ingeniously modified dialysis experiment (43).

Quantities of fresh (fluoride) blood were placed in small dialysers, and these were immersed in isotonic saline solutions containing percentages of dextrose varying between 0·05 and 0·2. After dialysis had been allowed to proceed for twenty-four hours at a low temperature, the percentage of dextrose in the dialysates was determined. In one of these no change was found to have occurred in the amount of dextrose originally present, but in the others this had either decreased or increased. It was found that the percentage of dextrose in the saline solution which showed no change was the same as that of the total sugar in the blood, as determined at the start of the experiment by the usual method. In this experiment the osmotic pressure of the

sugar in the blood was therefore found to correspond with the total amount of sugar present, thus indicating that the sugar must exist in blood in a free state. None of the objections brought forward against the dialysis experiments of other observers apply in this case. Suppose, for example, that some sugar was free and some combined in the blood. Between the two—*i.e.*, the free and combined—there would of necessity exist a certain equilibrium, so that a change in the amount of one would necessitate a change in the same direction on the part of the other. And when this blood was dialysed against a saline solution containing an amount of sugar equal to that present in the blood, there would have to occur a considerable diffusion of sugar into the blood in order to equalize the percentage of free sugar, and the total percentage in the blood would rise to a marked degree. The diffusion would have to proceed until the free dextrose in the blood equalled that of the solution outside the dialyser.

The conclusions which we may draw from these experiments is that the easily available sugar (dextrose) in blood exists therein in simple solution, and that there cannot be a loose protein-sugar compound, the sugar of which stands in an equilibrium with that which is free. But there is nothing in the experiments to indicate that there may not be, besides this free sugar, a certain amount that is locked away in some stable chemical compound, which is not readily broken, and with which the free sugar does not exist in a state of chemical equilibrium. In various ways it has been shown that this is the case, but it is very doubtful whether, in the circulating blood, any of this combined sugar can be utilized by the tissues.

This increase of the amount of free sugar in blood may occur under the following conditions :

1. Allowing the blood to stand for some time (fifteen minutes). Moderate warmth (58° C.) assists the process.

2. Treatment of the blood with the enzymes, emulsin, or invertin.

3. Boiling the blood with strong mineral acid, especially hydrofluoric acid.

Lepine designates the free sugar as *sucre actuel*, and the combined as *sucre virtuel*.

The extent of the increase in reducing power varies, however, according to which of the above methods is employed, being

much less marked with either of the first two than with the third. The following results demonstrate this :

Reducing Power per 1,000 Parts of Blood.*

	In Untreated Blood.	After Warming.
Fresh arterial (dog's)	1·33	1·50
After one hour at 58° C.	1·47	1·50
After allowing invertin to act on the blood	1·51	1·73

And to show the effect of treatment with acid† :

	In Untreated Blood.	After warming with Hydrofluoric Acid.	After boiling with Mineral Acid.
Arterial blood	0·740	1·510	—
Cardiac blood (average of twelve rabbits)	1·089	—	1·495
Cardiac blood (dog's)	0·860	—	1·300

Regarding the chemical nature of the compounds from which the *sucre virtuel* is split off, little that is definite can be said. Disaccharides and glucosides, including glycuronates, are probably responsible for the increase after treatment with invertin and emulsin, and possibly also for the spontaneous increase which occurs on allowing the blood to stand. Glycogen and dextrin may also account for some of the sugar, for the blood contains a strong diastatic ferment (glycogenase, see p. 147).

The much greater increase in sugar which occurs after boiling with acid may be due to disruption of a sugar group from proteins, certain varieties of which yield considerable quantities of reducing substance when thus treated. But the serum proteins appear to yield very little of this reducing substance, although egg protein yields a large amount (44). Polysaccharides, such as dextrin and glycogen, can scarcely be responsible for the increase due to acid, for they are very quickly hydrolyzed in blood, and their sugar must soon become free in drawn blood.

We must not leave this subject without taking notice of a very

* Lepine. † Pavy.

interesting observation to the effect that there is sometimes more *sucre actuel* in the blood of the carotid artery than in that of the right ventricle, indicating that in its passage through the lungs some *sucre virtuel* must be converted into *sucre actuel*, for of course the lungs cannot have added sugar to the blood (Lepine and Boulud, 45). It seems probable, if this observation be correct, that in the blood fresh from the liver there may be some polysaccharide (glycogen or dextrin) which soon becomes hydrolyzed by the powerful glycogenase of the blood.

For practical purposes, it is almost certain that we may disregard the *sucre virtuel*, more especially since, in the preliminary weighing and preparation of the samples of blood, sufficient time will have elapsed to convert it into *sucre actuel*. The reducing substance which appears after boiling with acid can be of no consequence in connection with sugar exchange between blood and tissues.

The Quantitative Relationship between the Sugar in Blood and that in the Urine.

There are two opposing views regarding this relationship—the one, that a strict parallelism exists, and the other, that none, or only a trace, of the sugar in the blood passes into the urine until a certain threshold value has been attained in the blood when an overflow into the urine occurs.

The simplest condition in which we can study this relationship is that dependent upon a constant overproduction of sugar in the organism. Such is most readily induced by stimulation of the great splanchnic nerve, which causes a marked and rapid transformation of the glycogen stored in the liver into dextrose, which escapes into the systemic blood, gradually raising the sugar percentage of this to a maximum, after which, as the store of available glycogen gets exhausted, it begins to fall. For some time after the nerve has been stimulated, no excess of reducing substance appears in the urine. The relationship between the percentage of reducing substances of the blood, and the amount and reducing power of the urine, is clearly demonstrated in the following curves (Curve I.) taken from a typical experiment (Macleod, 46).

Remarkably close agreement is evident between the curves of reducing power of blood and urinary outflow, but between

those of the reducing power of blood and urine there is only approximate parallelism ; the urinary curve does not begin to rise until that of the blood has attained a certain height, after which the two run more or less parallel, and during the decline the urinary curve falls more quickly than that of the blood. Under the conditions of the experiment, it may therefore be concluded that a demonstrable increase in the blood-sugar may occur before any overflows in the urine, after which there is approximate parallelism between the sugar of the blood and urine. This is, however, not usually the case, for in most cases of hyperglycæmia the sugar, once it has started to appear in the urine, quickly increases out of all proportion to that in the blood, thus indicating that the sugar is acting like a toxic substance.

To anyone who has carried out observations on the relationship of hyperglycæmia to glycosuria, it is very evident that much depends on the rate of urine formation. When this is slow, sugar may accumulate in the blood until quite a marked degree of hyperglycæmia has become established without any glycosuria, which, however, immediately develops, even with the slightest hyperglycæmia when there is diuresis. These facts are very clearly brought out in the following observations on rabbits injected with adrenalin (Pollak, 49) : Over 0·25 per cent. of sugar in the blood caused glycosuria whether urine formation was rapid or slow; with 0·15 to 0·25 per cent., glycosuria developed only when there was diuresis ; in a-glycosuric cases, with the latter degree of hyperglycæmia, the administration of a diuretic caused glycosuria.

Important observations have also been made on man, *e.g.*, after inducing moderate degrees of hyperglycæmia in comparatively healthy persons, either by administering adrenalin or by feeding with dextrose, Frank (47) observed that dextrose rarely appeared in the urine (Nylander test) until the percentage of sugar in the blood had risen above 0·2. Once this threshold was overstepped, however, the overflow of sugar into the urine became marked, and it continued for some considerable time after the percentage in the blood had commenced to fall, so that the maximum in the urine did not correspond to that in the blood, but occurred during the decline. These conditions were found to exist only in transitory cases of hyperglycæmia. In more persistent forms, such as occur in diabetes, the sugar threshold of the kidney may become altered, resulting either in

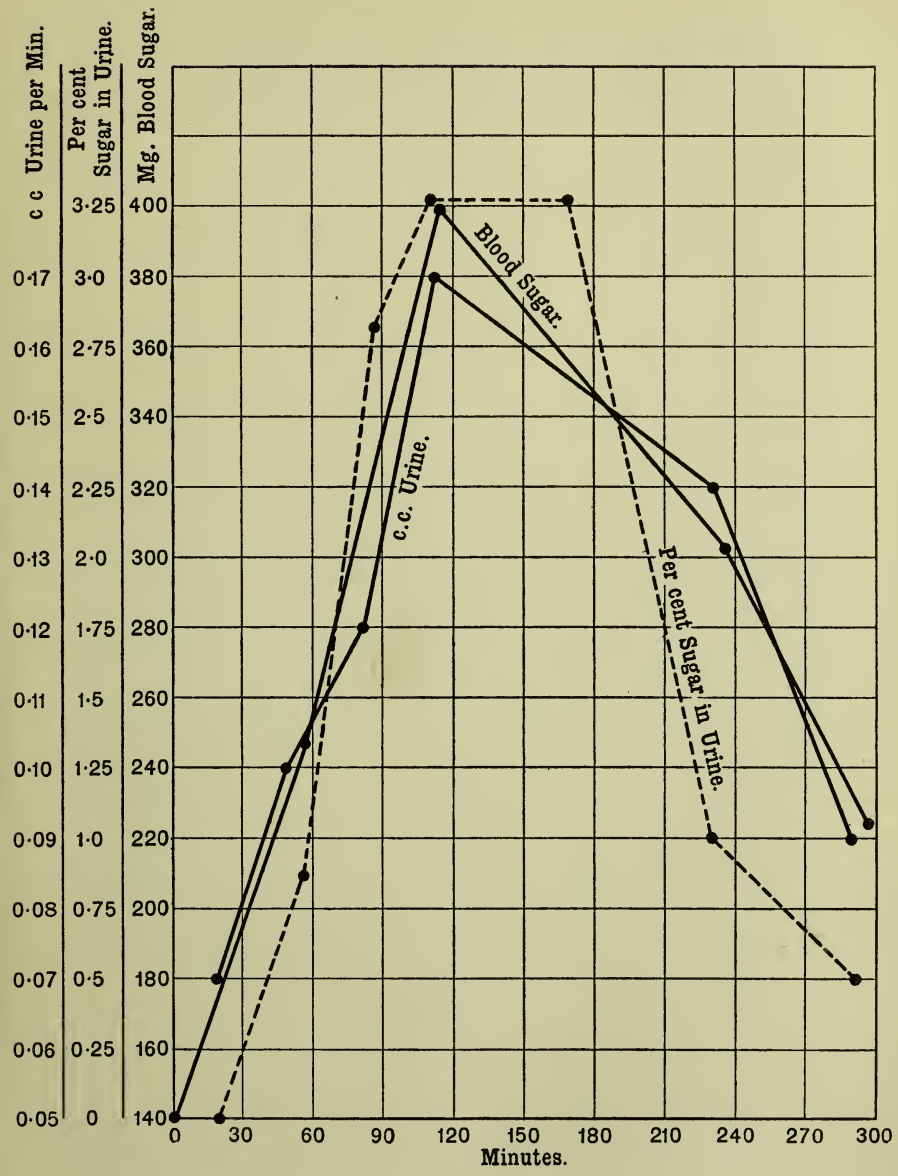

FIG. 1.

Curves showing the relationship between the concentration of sugar in arterial blood, the concentration in the urine, and the rate of urine formation, following stimulation of the splanchnic nerve.

its lowering, so that closer parallelism between the sugar of the blood and urine becomes established, or in its becoming raised, so that a marked hyperglycæmia exists without any glycosuria. This was observed in certain cases of chronic diabetes, and is well illustrated in the following table* :

Duration of Disease.	Sugar in Urine.	Sugar in Blood.
8 days	trace	0·105
7 months	—	0·110
4 to 5 years	—	0·152
,, ,, ...	—	0·163
,, ,, ...	trace	0·188
,, ,, ...	—	0·183
10 years	—	0·154
15 years	trace	0·224

It would be of great value to collect more data of this type, for, of course, the above are scarcely sufficiently numerous to justify any final conclusion. They are, however, of great value in the present connection, in that they show us that the urine does not reflect the reducing power of the blood, at least in chronic diabetes. Such results would point to the much greater value of determinations of the sugar of blood than that of the urine in such cases. By so doing the course of the disease can be accurately followed, and the effect of treatment watched. For example, in one case thus examined it was found that by rigid dietary control the sugar of the blood decreased from 0·183 to 0·107 per cent. The urine being sugar-free throughout, the only means other than an examination of the blood by which such a change in the sugar level of the blood could have been detected is a determination of the assimilation limit (see p. 197).

Glycolysis.

Since sugar is utilized by the tissues, there must be less of it in venous than in arterial blood. But even when these tissues are most active, the amount of dextrose utilized by them during each complete passage of the blood round the circulation is too small to make any measurable difference between the amounts in arterial and venous blood recognizable by the usual methods of analysis (*cf.* Magnus Levy, 50). This utilization of sugar is perhaps most simply studied in the case of an isolated

* Leifmann and Stern, 8.

preparation of the mammalian heart, about 4 milligrammes sugar per gramme of heart muscle being consumed by an ordinarily beating heart in one hour (56). This rate of consumption applies to the isolated dog's heart, supplied with blood containing an excess of sugar, and it may not be true for the heart of other animals, especially if the sugar content of the blood be normal. One observer (Camis, 56A) has stated that under these conditions no sugar disappears from the blood that is perfused through the heart of the cat, but that the glycogen stored in the heart muscle is used instead. The oxidation of dextrose in the tissues is probably preceded by a splitting of the dextrose molecule, and this preliminary *glycolysis* may occur, either in the blood or in the tissues. In order to observe it, we may measure from time to time the amount of free dextrose in blood or other tissue fluid kept at body temperature under aseptic conditions outside the body.

When blood is examined in this way, it has been found that, after a preliminary rise, the free sugar decreases, quickly at first, then more slowly. Thus in one experiment there was 0·111 per cent. sugar to start with, and only 0·068 after incubation for one hour (Lepine, 51). It is seldom, however, that such marked degrees of glycolysis are observed. Knowlton and Starling (56), for example, found that it amounted only to 0·01 per cent. in one hour. Laking of the blood destroys the glycolytic power; it can proceed under anaërobic conditions, but is accelerated by the presence of oxygen (Rona and Döblin, 52). The blood of different animals varies in glycolytic power, and this is said to become less after removal of the pancreas.

On the other hand, when serum alone is examined for glycolysis in the above manner, its sugar content does not change, thus indicating that it must be the formed elements of the blood to which the glycolysis is due. The leucocytes appear to be the responsible agents, for glycolysis occurs in suspensions of these in saline solution containing a certain proportion of phosphates.* That it is really glycolysis and not a condensation of dextrose that explains the decrease in reducing power in these experiments is evidenced by the fact that no increase of reducing power occurs when the incubated mixture is heated with acid. Paralactic acid also appears in the mixture (Levene and Meyer, 53).

* The importance of phosphates is also illustrated in the case of glycolysis produced by the action of H_2O_2 (Löb, 54).

When the tissues, or juices prepared from them, are similarly examined with regard to their action on glucose, a diminution in reducing power is likewise observed, but in this case the glucose has not been destroyed, but undergone a condensation, for when the incubated specimens are heated with acid, the original reducing power is restored. Certain mixtures of tissue juices, such as those of muscle and pancreas, appear to be more active in bringing about this condensation than when the juices are separately employed. Great uncertainty, however, prevails as to the exact significance of these results in explaining the mechanism of glycolysis in the intact organism, and the well-known work of Cohnheim and Hirsch, which suggested that glycolysis is dependent upon a proferment furnished by the muscles and activated by an internal secretion of the pancreas, does not seem to hold good (55). In most of these experiments of growth, micro-organism is thought to have been responsible for the glycolysis; antiseptics cannot be added because they inhibit the process.

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CHAPTER III

THE NERVE CONTROL OF THE SUGAR CONTENT OF THE BLOOD

We have seen that the concentration of sugar in the blood of the systemic circulation is maintained at a tolerably constant level—like the temperature and the tension of carbon dioxide, it is more or less a physiological constant. In all three cases the constancy represents a reciprocal relationship between production and loss. The chief source of sugar, so far as the blood is concerned, is the liver, and possibly also the muscles. In these places the sugar is stored away as glycogen, which can be delivered up again as sugar to the blood whenever required to maintain the concentration of sugar constant. Sugar is lost from the blood mainly by means of oxidation in the tissues, but a minute trace, as we have seen, may constantly escape in the urine. This escape of sugar from the kidneys may become marked when, as a result of failure in the adjustment between production and loss, there comes about an accumulation of sugar in the blood.

To make the fundamental conceptions quite clear, we may consider the following model: A tank contains water, which is kept at a constant level on account of a float which operates the valve of a supply-tube. There are two outlets from the tank, one wide, the other very narrow; and on the side of the tank, well above the level of the water, is a third outflow.

The tank represents the blood; the water in it, the blood-sugar; the inlet tube, the sugar-supply from the liver; the large outlet tube, the utilization of sugar by the tissue; and the narrow outlet tube, the constant leakage of sugar which occurs into the urine.* The float which operates the supply-valve represents

* *I.c.*, assuming for the present that the normal urine contains a trace of dextrose (see p. 10).

the mechanism, whatever it may be, which in the animal body adjusts the sugar-supply from the liver according to the amount of sugar in the blood. In such a model it is obvious that we may considerably alter the discharge through the larger outflow tube without disturbing the level of the water and without producing any considerable change in the volume discharging by the narrow outflow-tube. So in the animal, the consumption of sugar may vary enormously—for example, it may be greatly increased by muscular exercise—without there being any marked change in the level of the blood-sugar, or without any measurable change occurring in the percentage of dextrose normally present in the urine. This constancy of level evidently depends on the delicacy of the regulating mechanism, and there is evidence to show that it is not of so high an order, for when the consumption of sugar is excessive, a level lower than normal may be found in the amount of sugar in the blood (see p. 54).

Let us suppose now that some obstruction occurs in the large outflow-tube of our model, and that the regulator, although cutting down the supply-valve as far as it can, fails at last to stay the rise in the level. By an examination of the outflow through the narrow tube some inkling of the rise in level might be obtained; but since the increase of outflow by this tube could only be slight, the rise will, as a rule, go on unnoticed until the outlet higher up is reached, when the overflow will betray the raising of the level to this height. This, we believe, represents pretty accurately the conditions as we find them in glycosuria. The blood-sugar may, as we have seen, rise to quite a high level before there is any detectable glycosuria, but once this is established the blood-sugar overflows freely into the urine. The conditions existing in older cases of diabetes, where there is a continued rise in the blood-sugar level without glycosuria, would be represented in our model by a clogging up of the overflow outlet; and in phlorhizin-poisoning, where there is a constant leakage of sugar from the blood into the urine without any hyperglycæmia, by a widening in the narrow outlet-tube.

In order to form an estimate of the sensitiveness of the regulatory mechanism we must observe the constancy of the blood-sugar level from time to time. With a perfectly adjusted control this would vary but little, both when there is an excessive absorption of dextrose from the intestine and when tissue consumption of dextrose is at its highest. But, as we have seen,

the sugar-level of the blood varies considerably, not only in different animals, but also in the same one observed from time to time under perfectly constant conditions. When we observe its behaviour during the absorption of excessive amounts of carbohydrate, we usually find that the sugar level is raised, indicating that the assimilation limit has been overstepped. When amounts that are short of the assimilation limit are ingested, however, it has sometimes been observed (in man) that the opposite change occurs, thus indicating that a moderate increase in the sugar concentration in the portal blood may cause the liver to discharge less than its usual amount of sugar into the systemic circulation ; in one case, for example, the ingestion of 100 grammes of dextrose caused the percentage of sugar in the blood to fall from 0·13 to 0·09 (1).

Among the conditions which lead to hyperglycæmia, there are several, such as pain, emotion, and fright, which can scarcely be considered as pathological. The hyperglycæmia in these cases must indicate the necessity of a superabundant supply of sugar to the tissues, and it has been suggested that the object of this is that the sugar may be more readily available, so as to enable the animal, *e.g.*, when frightened, to guard itself against the offending agency, sugar being the foodstuff which is most readily oxidized in the development of muscular energy. Thus, when male cats are frightened by a barking dog they become glycosuric ; but female cats, on the other hand, who are indifferent to such treatment, remain a-glycosuric (Cannon, 2). When the muscles are voluntarily exercised, hyperglycæmia is not developed ; on the contrary—at least, in mild cases of diabetes—muscular exercise causes the sugar-level in the blood to fall.

Having formed some idea of the sensitiveness of the controlling mechanism, and also bearing in mind that a slight degree of hyperglycæmia is probably quite common and may be considered as physiological, we must next proceed to inquire into the nature of the mechanism by which variations in the blood-sugar level are brought about.

Referring again to our model, we see that the level of the water might be raised under the following conditions :

1. By the addition of water to the reservoir through other channels than the supply-tube, as by pouring water directly into it.

2. By an excessive supply through the supply-tube itself.

3. By an obstruction to the outflow in the wide outflow-tube.

In the animal the first of these conditions is illustrated by injecting sugar intravenously; the second by exciting the liver to increased sugar production; and the third by depressing the sugar-destroying or glycolytic power of the tissues.

The addition of sugar to the blood by intravenous injection is conveniently considered along with its administration in excessive amount by mouth, as a result of which we may imagine that such an excess is absorbed into the blood of the portal system, that it overwhelms the ability of the liver to fix it as glycogen, and so becomes added directly to the systemic blood. This we shall study later under the heading of Assimilation Limits (see p. 197).

Increased discharge of sugar from the liver is no doubt the commonest cause of most forms of hyperglycæmia—at least, of those that are transitory.

The substance in the liver from which the sugar is derived is glycogen. This polysaccharide is formed in the liver cell, being deposited therein out of sugar (dextrose) and certain other substances carried to the liver in the blood coming from the intestine. Disregarding for the present the nature of the process by which glycogen is formed—its sources and the process of its formation in the liver cell—and assuming that the liver is well supplied with it, we shall proceed to a study of the conditions which so govern its reconversion into sugar that this occurs just in proportion to the needs of the blood. It will be convenient to designate this process of sugar formation from glycogen the glycogenolytic process.

In order to reveal the nature of a normal process, it is necessary for us to examine the effects which follow some experimental interference with it. The normal function of the vagus on the heart-beat is revealed by severing the continuity or by stimulating the nerve, and from the results we draw our conclusions regarding the function of the nerve in the intact animal. And so with the glycogenolytic function of the liver: to understand the nature of the mechanism by which it is controlled we must bring about experimental interference with it and observe how it behaves. Referring to our model, we may say that our present problem is to find out the nature of the float which operates the valve on

the supply-pipe. That is, we must seek for the agency which acts between the amount of sugar in the blood and the sugar-producing function of the liver in such a manner as to keep the former more or less constant in level.

Two possibilities exist—(1) that it is a nervous reflex, and (2) that it is dependent upon the composition of the blood acting directly on the liver cell. We will consider first the evidence in favour of the former possibility.

Nerve Control.

It is well known that the amount of sugar in the blood is subject to nerve control. The occurrence in man of glycosuria after wounds to the nervous system or irritation of sensory nerves, its occurrence as a symptom in some cases of brain tumour, and the undoubted association between neurotic conditions and diabetes, are of interest as indicating the relationship between the nervous system and disturbances in the glycogenic function. But these facts do not necessarily imply that there is a direct nerve control of the glycogenolytic activities of the liver ; they might equally well be dependent upon the nerve control of some ductless gland which secretes substances into the blood capable of affecting the glycogenolytic process, or they might depend on a change in the blood-supply of the liver.

For the present, therefore, we must examine the experimental evidence that there is a direct nerve control of the glycogenolytic process. The form of experimental glycosuria which has usually been considered to furnish the strongest evidence for such a control is piqûre, or puncture of the fourth ventricle. In the rabbit and dog this is invariably followed by hyperglycæmia (and glycosuria), provided the liver contains glycogen. I need not occupy our time with an account of the many experiments which illustrate this (3). Instead of doing so, we shall devote our attention to the question as to whether the results of piqûre are really due to a direct nerve stimulation of the glycogenolytic activities of the hepatic cell, or whether they may not be secondary to some other change which can influence this. The following possibilities exist :

1. That the hyperglycogenolysis is due to an increased blood-supply to the liver, on account of dilatation of the intrahepatic

vessels. In this connection we must also think of a rise of temperature in the blood.

2. That it is the result of asphyxia.

3. That it is due to a hypersecretion of adrenalin into the blood.

So far as I know, no exact observations have been made on the extent of the vascular changes following piqûre, but no doubt they are profound. Bernard recognized a dilatation of the vessels, and it is apparent to anyone who has performed the piqûre experiment. The thermogenic centre is also irritated by the puncture, and a rise in the blood-temperature, amounting sometimes to several degrees, has been observed. This, along with the increased blood-supply to the liver, might conceivably be the cause of the hyperglycogenolysis, by inducing greater action of the diastatic ferments of the blood on the glycogen in the liver. After giving nicotin to rabbits in sufficient dosage to produce a block in the sympathetic ganglia, piqûre does not cause glycosuria (Macleod and Dolley), but this is not in itself proof of the existence of nerve control, for the absence of the glycosuria may be due to a faulty excretion of sugar by the kidney on account of the low blood-pressure (see p. 46).

Turning now to the effect of piqûre on the respiratory mechanism, we find that the disturbance is so great that it cannot be more than approximately corrected. Henderson and Underhill (4) have found that piqûre produces a marked diminution in the percentage of carbon dioxide in the blood, because of the excessive breathing which immediately follows the operation.* These authors do not state that it is because of this "acapnia" that the glycosuria occurs, but they point out that such a condition exists, not only in piqûre, but also in other forms of glycosuria.

After performing piqûre on etherized rabbits, it was attempted to prevent the acapnia by causing the rabbits to breathe in an atmosphere which contained an excess of carbon dioxide; and in the few experiments in which, by blood-gas analysis, it could

* The disturbance of the respiratory centre as a result of piqûre causes, first of all, a hyperpnœa, which is responsible for the washing out of the carbon dioxide of the blood, and therefore the subnormal breathing soon follows.

be shown that this had been accomplished, it was also noted that the polyuria was absent, and that the glycosuria was later in appearing than is usually the case.

It must be remembered, however, that glycosuria also accompanies the opposite condition to acapnia—namely, that in which there is an increase in the amount of carbon dioxide in the blood (*e.g.*, in asphyxia ; see p. 177)—consequently the only conclusion which we can draw is that, with a disturbance in the carbon dioxide of the blood in either direction, there is an associated derangement of the glycogenic function of the liver.

Within the past few years the view has been advanced that the direct cause of the hyperglycæmia which follows piqûre is a hypersecretion of adrenalin into the blood. If such should prove to be the case, then, of course, we should have to consider even this, the most typical of the so-called nervous forms of glycosuria, as really dependent on hormone influence, and we should be encouraged to expect that the other forms of hyperglycæmia which are associated with disturbances of the nervous system are also more or less dependent upon the presence of hormones in the blood. It becomes very important for us at this stage, therefore, to settle the question, as far as we can, in the case of the adrenal gland. We may consider it as a test case. There are several facts which, taken together, would seem to justify the above view. In the first place, it is well known that hyperglycæmia occurs when there is an excess of adrenalin in the blood. Although we shall discuss the nature of this form of experimental glycosuria elsewhere, it may be well for the present to note that in glycogen-rich animals the subcutaneous or intraperitoneal injection of 1 milligramme per kilogramme body weight of adrenalin chloride usually results in the production of hyperglycæmia, accompanied by polyuria and glycosuria. In animals that have been rendered glycogen-free, as by the administration of strychnine, although the adrenalin does not cause hyperglycæmia, it nevertheless brings about a marked disturbance in carbohydrate metabolism (5), as evidenced by the fact that glycogen becomes deposited in the liver. On the other hand, when there is a deficiency of adrenalin in the blood, as occurs when the adrenal glands are excised (in dogs), and in Addison's disease (in man), it has been found that a marked diminution in the reducing power of the blood follows (6). The moribund condi-

tion of dogs after adrenalectomy may, however, account for the hypoglycæmia. After adrenalectomy in rats, the liver becomes glycogen-free, but this is not the case in rabbits (6A).

The above view assumes that the adrenal gland secretes adrenalin into the blood, and that this secretion is under nerve control. Let us see whether such an assumption is warrantable by the facts. There are four more or less reliable physiological tests for the presence of minute quantities of adrenalin in blood: (1) Its mydriatic (dilatory) action on the pupil of the excised eye of the frog; (2) its effect in raising the arterial blood-pressure; (3) its power of lessening the rhythmic contractions of the longitudinal coat of the intestine immersed in Ringer's solution; (4) its stimulating action on the rhythmical contractions which occur in the excised uterus of the virgin rabbit immersed in oxygenated Ringer's solution. But these tests for the presence of adrenalin in blood must be interpreted with caution. The results of one test alone should not be depended on, but two at least should be employed. For this purpose the intestine and uterus are probably the most suitable, and the results should not be considered as positive unless a definite adrenalin reaction is given by both. The reason for such precautions is that the blood may contain substances that simulate the adrenalin action when only one test is employed, but this is not likely to be the case with two, especially when the adrenalin effect on these is opposite in nature, as is the case when the intestine and uterus are chosen. Blood-serum also contains substances which inhibit the adrenalin action (7). Thus a much lower percentage of adrenalin in Ringer's solution gives the above reactions than is the case when the adrenalin is present in blood-serum.

By the employment of one or other of the above methods, several investigators have concluded that, under normal conditions, the blood of the suprarenal vein contains distinctly more adrenalin than the blood from other parts of the body (8). On the other hand, by using the uterus and intestine tests, Stewart was unable to detect adrenalin in the blood issuing from the suprarenal veins in etherized, but otherwise normal dogs.

Whether or not detectable quantities of adrenalin are present in normal blood, there is no doubt that it is present when the nerve-supply to the gland is stimulated. This nerve-supply is through the corresponding great splanchnic, and Stewart has been able to confirm the observations of previous workers, that

when this nerve is stimulated adrenalin is present in easily detectable amount in the blood of the suprarenal veins (9). The increase is sometimes quite marked ; it can, for example, cause the blood-pressure (in the same animal) to rise after the abdominal viscera have been removed (10). Evidence of stimulation of the adrenal activity is also said to be obtainable in piqûre ; thus, blood from the auricular vein, following the operation, in rabbits exhibits mydriatic properties not possessed by normal blood from the same animal. Other things, such as hypertonic salt solution, may, however, have like effect, and some observers have been unable to confirm the observation (8A). There is also histological evidence that the adrenal gland is stimulated by piqûre. When, in rabbits, one gland is removed, and piqûre then performed, the medullary cells of the remaining gland, after glycosuria has developed, will, when compared with the normal one, be found to stain less deeply, and to contain fewer granules. Its bloodvessels are also dilated. When neither gland is removed, but the left splanchnic nerve is cut, piqûre causes the above changes only in the gland on the right side (Kahn, 11). The drug diuretin stimulates the glycogenic centre, and produces the same effects as piqûre ; thus, it does not cause hyperglycæmia after ablation of the adrenal glands or after splanchnotomy (Nishi, 8A).*

The following facts may therefore be accepted as established : (1) That adrenalin causes a stimulation of the glycogenic function, and (2) that the secretion of adrenalin is under nerve control. They encourage us to consider the possibility that the action of the nervous system on the glycogenic function of the liver is dependent upon its control of the secretion of adrenalin. Bearing on this possibility, the following observations have so far been made :

Mayer (12) found that after removal of the adrenal glands piqûre caused no glycosuria in rabbits ; in three dogs that survived for some time, the operation was not followed by the usual hyperglycæmia. Wertheimer and Babbery (13) were,

* An interesting fact regarding the innervation of the adrenal glands in the rabbit has been brought to light by the experiments of Kahn and Nishi above referred to. This is that the left splanchnic nerve furnishes the nerve-supply of both adrenals. After section of this nerve alone diuretin therefore causes no hyperglycæmia, although it does so after section of the corresponding nerve on the right side, even after all nerve fibres connecting the left splanchnic with the left adrenal have been severed.

however, unable to confirm these results in the case of cats, for after adrenalectomy piqûre still produced glycosuria.

It is evident, however, that the observations on piqûre are too involved, on account of the serious disturbances of respiration and circulation, to enable us to conclude whether the glycogenolysis is due to a hypersecretion from the adrenal gland. We must look for a simpler form of experimental hyperglycæmia in which we can study this possible relationship. Such a form of hyperglycæmia is that caused by stimulation of the great splanchnic nerve. This is most conveniently done on the left side, on which the nerve is readily accessible without much loss of time, an important desideratum in all experiments in which comparisons are to be made in the reducing power of the blood. The following table shows the effect produced

PERCENTAGE OF SUGAR IN ARTERIAL BLOOD.*

	Before Stimulation of the Splanchnic Nerve.	In from ½-2 Hours after Stimulation.
Average ..	0·159	0·280
Minimum ..	0·128	0·198
Maximum ..	0·180	0·378

Similar observations, but in which O_2 was inspired, gave :

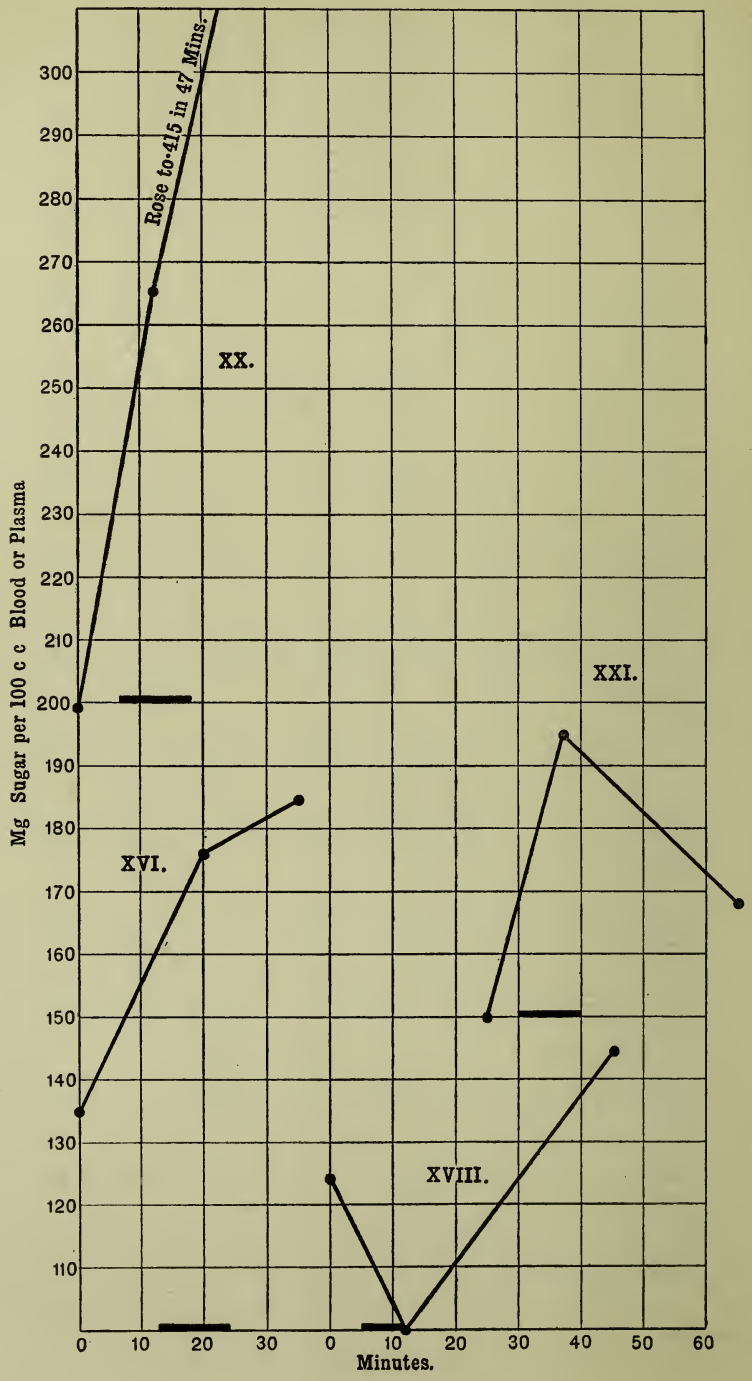
Average ..	0·180 (only three observations)	0·213 (eight observations)
Minimum ..	0·151	0·114 (?)
Maximum ..	0·231	0·255

PERCENTAGE OF SUGAR IN THE BLOOD FROM THE VENA CAVA.

	Before Stimulation of the Splanchnic Nerve.	In from 5-10 Minutes after Stimulation.
Average ..	0·148 (eight observations)	0·190 (eight observations)
Minimum ..	0·111	0·110
Maximum ..	0·199	0·280

on the reducing power of the blood or blood-plasma, taken either from the femoral artery or from the vena cava opposite the liver, by stimulation of the nerve, when the adrenal glands are intact (14). The influence of oxygen inhalations is incidentally shown, and it is worthy of note that intratracheal insufflation of this gas distinctly diminishes the hyperglycæmia, although it does not prevent it, as is the case during stimulation of the central end of the vagus nerve (see p. 79). The results which most clearly demonstrate the profound effect of splanchnic stimulation on sugar production by the liver are those in which the blood of the vena cava was examined. The following curves, plotted from the results of seven such experiments, very clearly

* This table is a summary of the fuller tables quoted in references (14).


Rose to 415 in 47 Mins.
XX.
XXI.
XVI.
XVIII.
Mg Sugar per 100 c c Blood or Plasma
300
290
280
270
260
250
240
230
220
210
200
190
180
170
160
150
140
130
120
110
0
10
20
30
0
10
20
30
40
50
60
Minutes.

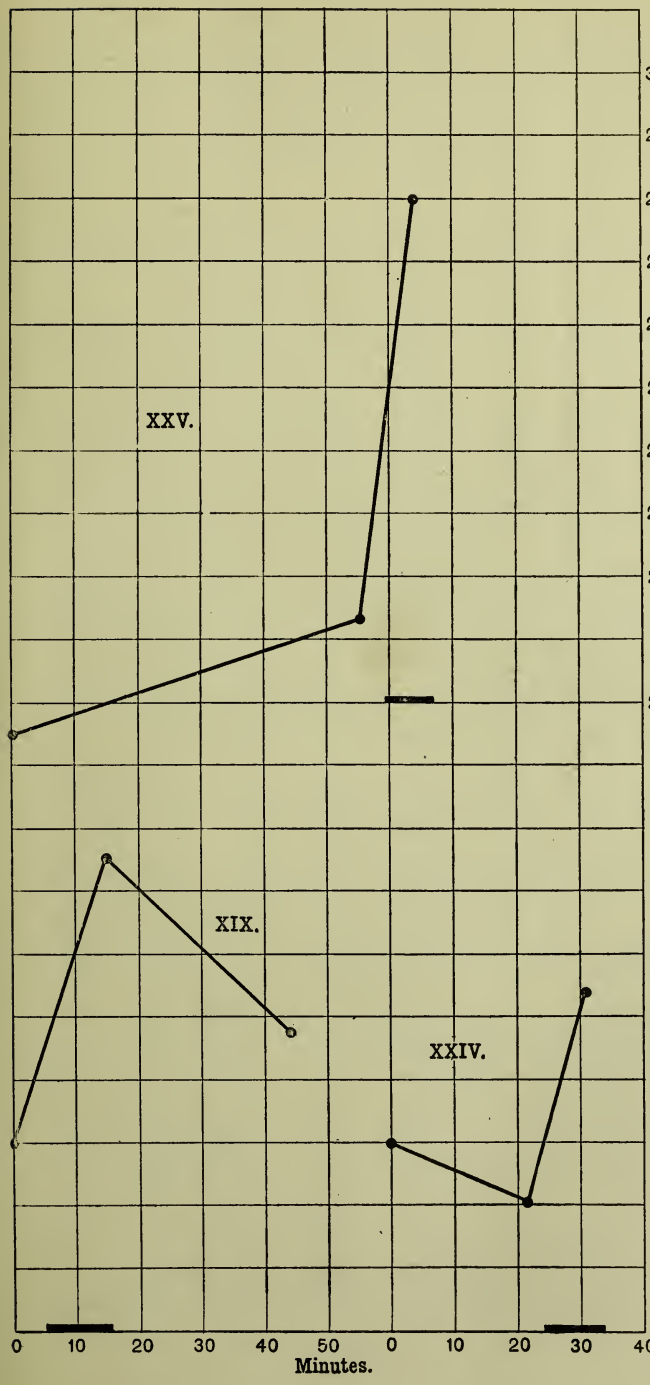


Fig. 2.—Curves compiled from seven experiments showing increase of sugar in blood of inferior vena cava produced by stimulation of the (left) splanchnic nerve. The thick horizontal lines indicate the duration of stimulation in each case.

show this. Observations made on the arterial blood, although perfectly definite in their results, are open to the criticism that a considerable lapse of time occurs between the beginning of stimulation and the collection of the blood-samples, during which time changes in other parts of the organism may have been occurring.

Turning now to the result of stimulation of this nerve when one or both adrenal glands are removed, I have found, in association with Pearce and Christie, that after excision of the gland on the left side in dogs stimulation of the great splanchnic nerve on the same side fails to cause any immediate increase in the percentage-reducing power of the blood or blood-plasma taken from the vena cava.* In these experiments the removal of the gland was conducted as carefully as possible, so as to avoid injury to the splanchnic nerve, which lies under it, although it is probably impossible to avoid a certain amount of injury, on account of the presence in the medulla of the gland of certain of the sympathetic fibres which seem to grow into it during its embryological development. However, the rise in arterial blood-pressure following stimulation of the splanchnic nerve demonstrated that the removal of the adrenal in our experiments had not damaged more than a few of the splanchnic fibres.

The following table and curves (Fig. 3) give the results :

PERCENTAGE OF SUGAR IN THE BLOOD OF THE VENA CAVA, WITH LEFT ADRENAL REMOVED.

	Before Stimulation of the Left Splanchnic Nerve.	After Stimulation† of the Left Splanchnic Nerve.
27	0·118	0·124 (*10*)
	—	0·109 (*25*)
28	0·120	0·101 (*5*)
	0·082 (thirty minutes later)	0·103 (*20*)
29	0·171	
	0·173 (thirty minutes later)	0·177 (*16*)
30	0·163	—
	0·127 (thirty minutes later)	0·113 (*15*)
31	0·249	0·270 (*6*)
	0·248 (twenty-five minutes later)	0·256 (*20*)
49	0·242	0·225 (*12*)

* For brevity's sake we shall hereafter designate this " cava blood."

† The figures in italic indicate the number of minutes elapsing between the removal of the various samples of blood. In order, the figures are to be read from left to right.

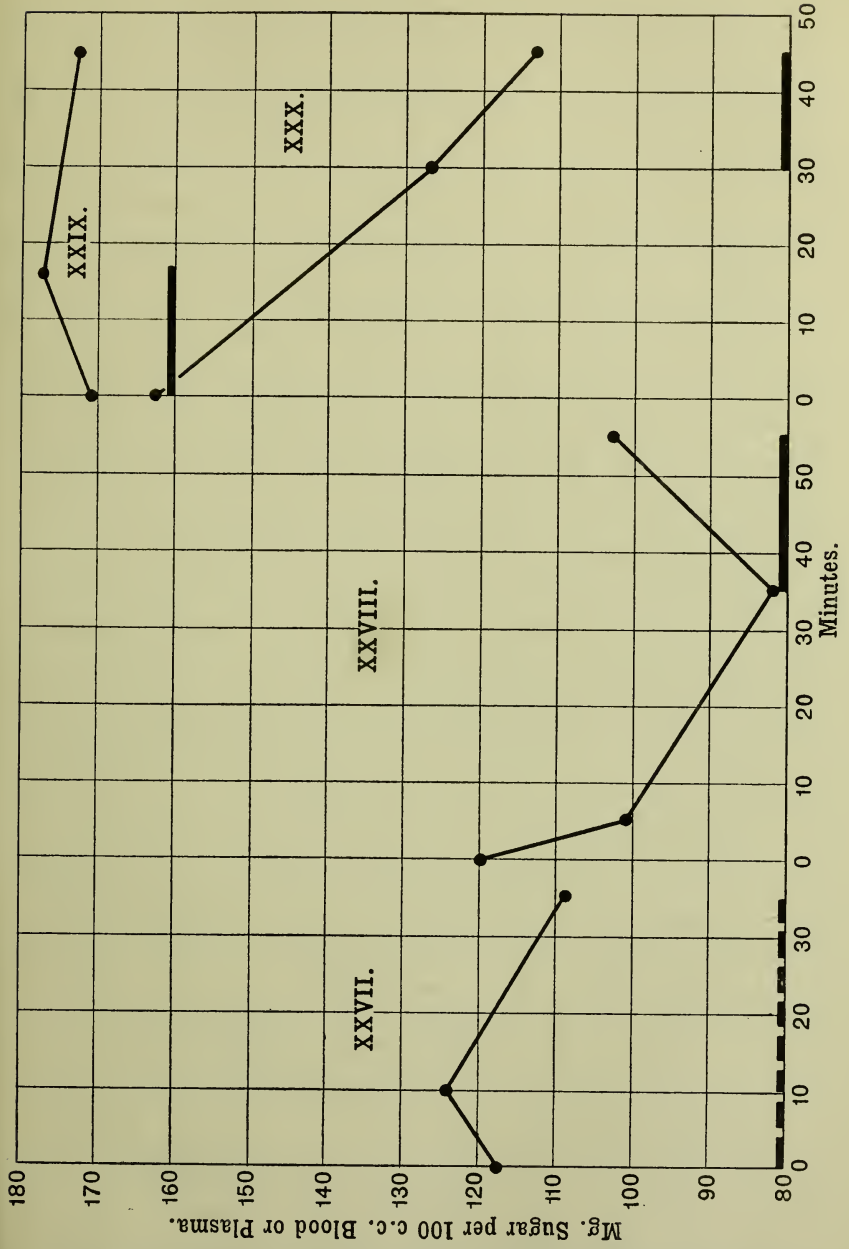


FIG. 3.—Curves compiled from four experiments showing behaviour of sugar of cava blood following stimulation of splanchnic nerve *after adrenalectomy*. Horizontal dark lines indicate duration of stimulation.

Although similar results, with regard to glycosuria, have been obtained by Gautrelet and Thomas (16), they do not in themselves indicate that an increased secretion of adrenalin is responsible for the hyperglycæmia which is produced by stimulation of the splanchnic nerve. It is obvious, if such is the case, that section of the nerve path between the adrenal glands and the liver, as by cutting the hepatic plexus, should be followed by the usual degree of hyperglycæmia when the splanchnic nerve is stimulated, the adrenal glands on both sides being intact.

In the first experiments of this nature which we performed, somewhat irregular results were obtained : there was sometimes evidence of hyperglycogenolysis and sometimes not. It was thought that the irregularity might be due to incomplete section of the various fibres that compose the hepatic plexus, for although the bulk of these had unquestionably been severed—for everything composing the hepatic pedicle save the portal vein itself had been cut—there might yet remain some isolated fibres on the outer coat of the portal vein. In the later experiments this possibility was guarded against by making linear sears, interdigitating with one another, on the portal vein. After thus completely denervating the liver, it was found that only rarely was splanchnic stimulation followed by hyperglycogenolysis, and that when such occurred, this was to a lesser degree than usual.

I shall give, in illustration, the results obtained after the more thorough denervation. They are as follows :

Percentage-Reducing Power of Cava Plasma after Complete Section of the Hepatic Plexus.

No. of Experiment.	Before Stimulation of the Splanchnic Nerve.	From 3 to 16 Minutes after Stimulation.
74	0·241	0·259 (16 minutes)
75	0·254	0·268 (3 ,,)
	0·232*(20min.)	0·254 (3 ,,)
76	0·129	0·134 (6 ,,)
	0·156 (20 min.)	0·154 (5 ,,)
And using " blood " instead of plasma—		
65	0·130	0·129 (10 minutes)
68	0·188	0·182 (6 ,,)
	0·179 (20 min.)	0·181 (5 ,,)

The results of these observations on the cava blood are in harmony with older ones of a similar nature, in which the arterial

* Time elapsing since first stimulation.

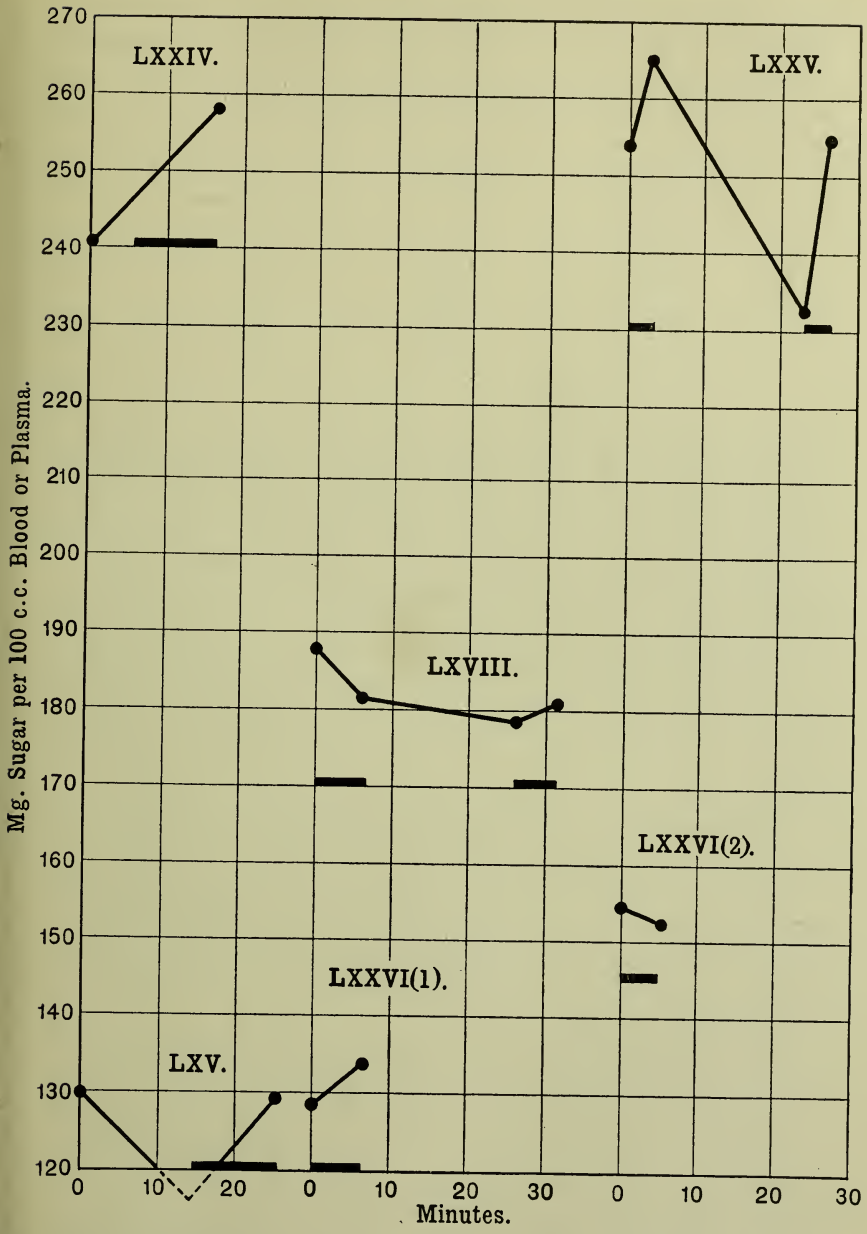


FIG. 4.—Curves compiled from five experiments showing behaviour of cava blood, or plasma, following stimulation of splanchnic nerves *with hepatic nerves cut*. Horizontal dark lines indicate duration of stimulation.

blood was examined (17), but in which the outer coat of the portal vein was not seared. This is shown in the following table :

PERCENTAGE-REDUCING SUBSTANCE IN ARTERIAL BLOOD OF DOGS WITH HEPATIC PLEXUS CUT.

Before Stimulation of the Splanchnic Nerve.	After Stimulation of the Splanchnic Nerve.	Minutes after Stimulation applied at which Blood was removed.
0·162	0·189	90
0·191	0·162	40
—	0·229	90
0·171	0·184	55
0·184	0·183	50
—	0·164	110

The conclusion is that it cannot be merely because of an increased secretion of adrenalin into the blood that stimulation of the splanchnic nerve produces hyperglycæmia. We must look for some other explanation of the results, and the only one possible is that the glycogenic function must be under a direct nerve control, which is, however, dependent on the presence of a certain amount of adrenalin in the blood.

If such is the case, stimulation of the hepatic plexus should be capable of producing hyperglycæmia in animals in which the adrenal glands are intact, but not so in those from which these have been removed. The following observations bear on this question :

In order to stimulate the hepatic plexus we have not attempted to dissect out the individual fibres of the plexus, but we have stimulated the tissues in which they lie by laying these on guarded electrodes. By this procedure the electrical current may, of course, be partly short-circuited through the tissues without really stimulating the nerve fibres. A strong current must therefore be used.

The first observations on the effect of such stimulation with intact adrenals were made some years ago on the arterial blood collected partly before stimulating the nerves, and then, again, at periods varying from one to two hours, after beginning the stimulation. The results are given in the table on p. 69.

The only negative result is that obtained in the last experiment, in which it is probable that the electric current did not affect the nerve fibres, for the electrodes, instead of being tied in position on the pedicle, were applied by hand. A possible

objection that can be made to these experiments is that considerable periods of time elapsed between the first application of the stimulus and the collection of the samples of blood, and that during this period a spontaneous (ether) increase in the reducing power of the blood had occurred. This is, however,

PERCENTAGE-REDUCING POWER OF ARTERIAL BLOOD.

Before Stimulation of Hepatic Nerves.	After Stimulation of Hepatic Nerves.
0·103	0·231 (50 minutes)
—	0·231 (90 ,,)
—	0·325 (60 ,,)
—	0·360 (90 ,,)
0·210	0·264 (60 ,,)
—	0·303 (120 ,,)
0·118	0·117 (60 ,,)
—	0·099 (99 ,,)

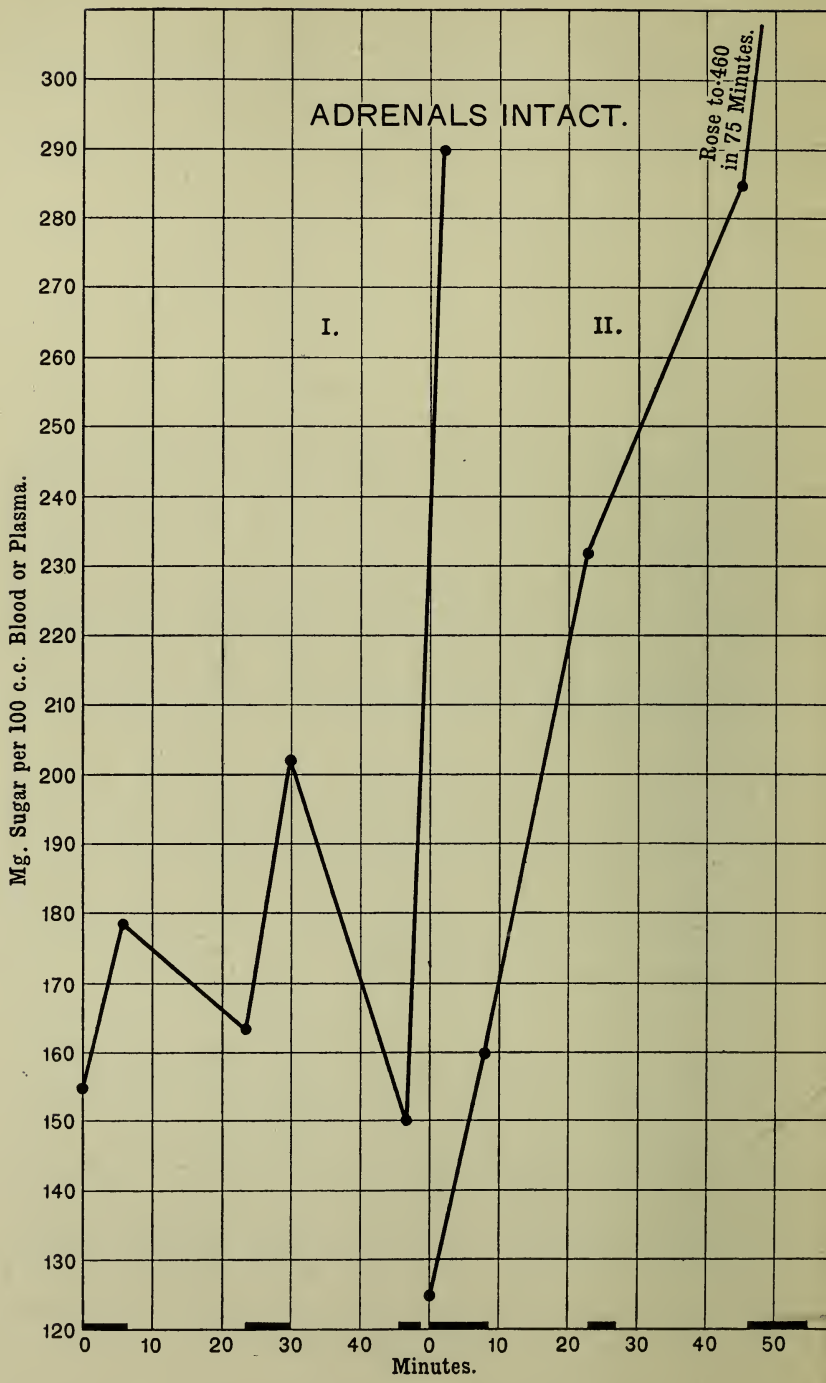
highly improbable, because oxygen was administered by intra tracheal insufflation, and, in other experiments in which the same time intervals were used, no increase in blood-sugar was observed.

However, in light of the great importance of these observations in connection with the present question, we have repeated them, but instead of taking the arterial blood collected some time after the stimulation, we have taken that of the vena cava a few minutes after. The results of such experiments are as follows:

THE EFFECT ON THE REDUCING SUBSTANCE OF THE BLOOD-PLASMA OF THE VENA CAVA INFERIOR PRODUCED BY STIMULATION OF THE HEPATIC NERVES WITH THE ADRENALS INTACT.

Reducing Substance per Cent.		Change produced. (Figs. = per cent. increase).	Remarks.
Before Stimulation.	After Stimulation.		
0·153	0·179 (*6*)*	17	Coil, 6 centimetres. Pedicle uncut. Rise in blood-plasma following stimulation.
0·165 (*17*)	0·204 (*7*)	23·6	Pedicle cut. Rise in blood-plasma now very slight. Coil, 4 centimetres.
0·150 (*16*)	0·294 (*6*)	94	—
0·125	0·160 (*8*)	20	Coil, 4 centimetres. Pedicle uncut during first half of experiment. Stimulation produced rise in arterial blood-plasma so long as pedicle uncut.
0·233 (*15*)	0·243 (*3*)	4	
0·292 (*20*)	0·372 (*8*)	27·3	
0·425 (*14*)	0·472 (*6*)	11	

* See footnote on p. 64.


ADRENALS INTACT.
I.
II.
Rose to 460 in 75 Minutes.
Mg. Sugar per 100 c.c. Blood or Plasma.
300
290
280
270
260
250
240
230
220
210
200
190
180
170
160
150
140
130
120
0
10
20
30
40
0
10
20
30
40
50
Minutes.

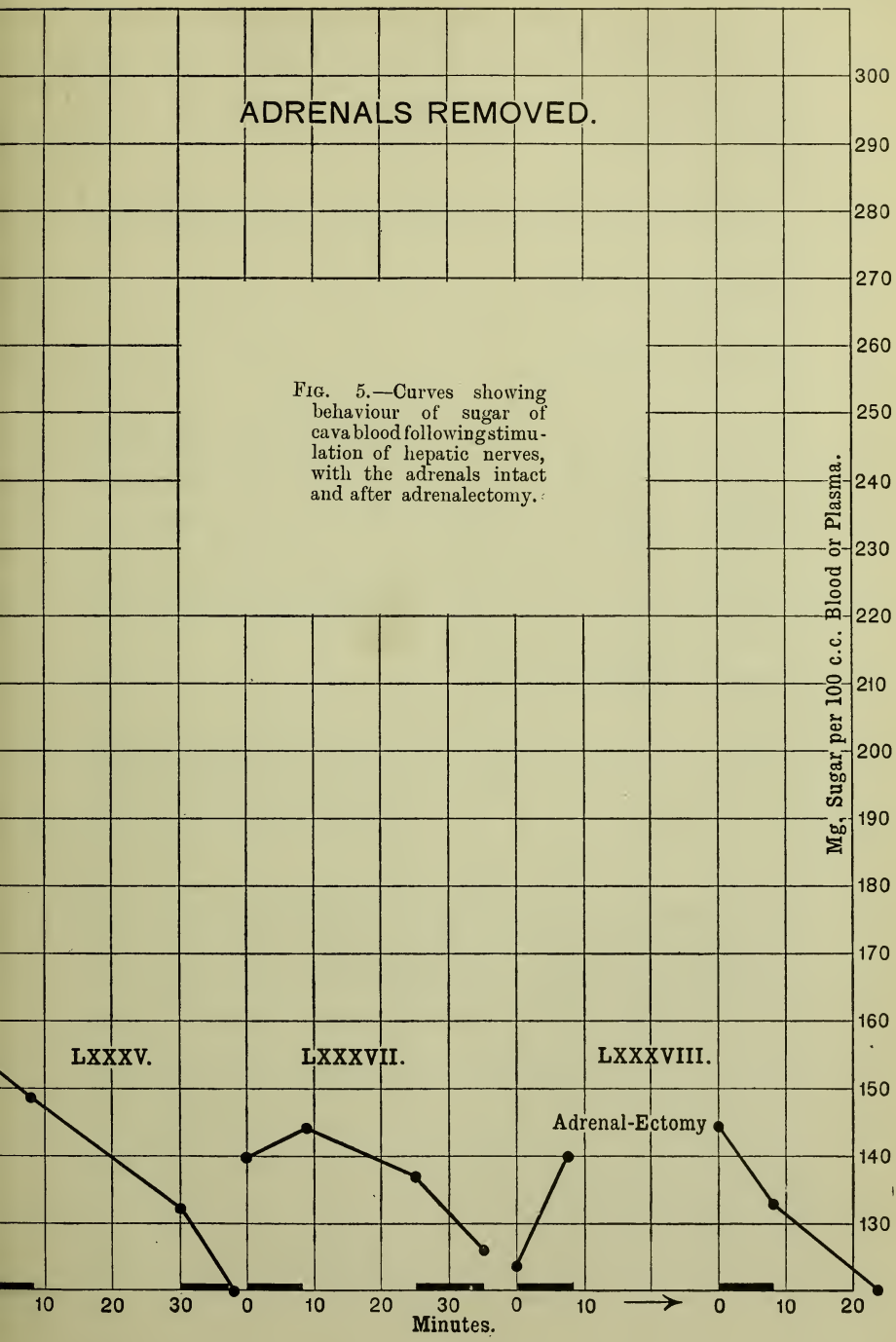


FIG. 5.—Curves showing behaviour of sugar of cava blood following stimulation of hepatic nerves, with the adrenals intact and after adrenalectomy.

Stimulation of the hepatic nerves therefore produces a striking hyperglycæmia within a few minutes, whether the fibres central to the point of stimulation are cut or uncut. On the other hand, such stimulation has no effect in the absence of the adrenal glands, as is evident from the following experiments :

THE EFFECT ON THE REDUCING SUBSTANCE OF THE BLOOD-PLASMA OF THE VENA CAVA INFERIOR PRODUCED BY STIMULATION OF THE HEPATIC NERVES AFTER REMOVAL OF BOTH ADRENAL GLANDS.

Reducing Substance per Cent.		Change Produced.	Remarks.
Before Stimulation.	After Stimulation.		
0·155	0·149 (*8*)*	Fall	Coil, 6 centimetres. Stimulation did not affect blood-pressure.
0·133 (*22*)	0·120 (*8*)	,,	Blood-pressure, 50 millimetres.†
0·323‡	0·274 (*8*)	,,	—
0·239 (*14*)	0·225 (*8*)	,,	Blood pressure, 65 millimetres.
0·141§	0·145 (*9*)	No change.	—
0·137 (*16*)	0·126 (*10*)	Fall	—
0·124‖	0·140 (*8*)	13 per cent. rise	Blood used in first two, plasma in others. Animal very sensitive to ether.
0·144¶	0·133 (*8*)	Fall	—
—	0·110 (*25*)	,,	—
0·344**	0·291 (*10*)	,,	—
0·230 (*20*)	0·234 (*10*)	No change	—
0·156‖	0·194 (*10*)	24 per cent. rise	Coil, 4 centimetres. After the adrenalectomy the removal of blood caused a fall to 70 millimetres Hg in blood-pressure. The blood in cannula was also partly clotted.
0·273 (*43*)¶	0·400 (*10*)	46 per cent. rise	
0·179	0·157 (*10*)	Fall	—

The figures in the two preceding tables are plotted in curves in Fig. 5, and in these the influence of adrenalectomy on the excitability of the hepatic nerve control is very clearly seen.

The importance of the adrenal glands in maintaining the

* The figures in italic indicate the number of minutes elapsing between the removal of the various samples of blood. In order, the figures are to be read from left to right.

† Unless otherwise noted, the blood-pressure was between 90 to 120 millimetres Hg.

‡ Thirty minutes after adrenalectomy. Coil, 6 centimetres. Stimulation did not affect blood-pressure.

§ Thirty minutes after adrenalectomy. Coil, 4 centimetres. Stimulation did not affect blood-pressure.

‖ Adrenals intact. ¶ Adrenals removed.

** High because splanchnic nerve had been stimulated earlier in experiment.

sugar concentration in the blood has also been shown by Porges (16), who found that their total removal in dogs was followed by a progressive hypoglycæmia, and that their disease in man (Addison's disease) is accompanied by the same condition. Mayer (18) and Frouin (19) likewise found that the glycosuria and polyuria of depancreated animals became distinctly less after adrenalectomy. Such observations are, however, not very convincing because of the moribund condition of the animal produced by the double operations. This in itself might depress the kidney function (see p. 46). Mayer has, however, examined the amount of blood-sugar in three depancreated dogs that survived cauterization of the adrenals for an hour, and has found, in two of these, a distinct decrease.

We may conclude that in the entire absence of adrenalin from the blood it is impossible to excite hyperglycogenolysis by stimulation of the nerve-supply to the liver (piqûre, splanchnic stimulation, hepatic nerve stimulation). The only exception to this general statement, so far as is known, exists in the case of the hyperglycæmia produced by "diuretin," a drug which is believed to stimulate the glycogenic centre (Nishi), and which continues to cause hyperglycæmia in adrenalectomized rabbits.*

We do not know how much adrenalin must be present in the blood in order that the functional integrity of the nerves may be maintained. When the adrenal veins are ligated on one side, splanchnic stimulation can still result in hyperglycæmia ; but this is not usually the case when these are ligated on both sides. The Table on p. 74 illustrates this.

Tying the vein on the left side alone did not render stimulation of the corresponding splanchnic nerve ineffective, but when the veins on both sides were tied, it was found, in the majority of cases, that such stimulation had no longer its usual effect—*i.e.*, it produced only a mild degree of hyperglycogenolysis or none at all. Of the three observations in which a rise did occur, two can probably be discounted because they were made on animals from which a considerable amount of blood had already been removed, and in which the arterial blood-pressure was abnormally low. We have invariably found that the results under such conditions are inconstant and unreliable (see p. 192).

* There is, however, still some doubt regarding the exact mode in which diuretin acts in producing hyperglycæmia (*cf.* Miculicich, *Archiv f. exp. Path. u. Pharmac.*, 1912, vol. lxix., p. 128).

This leaves one result only in which splanchnic hyperglycogenolysis persisted after tying the veins.

There is evidently an important difference between the sympathetic innervation of motor mechanisms, such as the dilator pupillæ and the heart-beat and that of the glycogenic function, for the former retain their activity in the absence of

THE EFFECT ON THE AMOUNT OF REDUCING SUBSTANCE IN THE BLOOD-PLASMA OF THE VENA CAVA INFERIOR PRODUCED BY STIMULATION OF THE LEFT SPLANCHNIC NERVE, AFTER LIGATION OF THE ADRENAL VEINS.

	Reducing Substance per Cent.		Change produced. (Figs. = per-centile rise.)	Remarks.
	Before Stimulation.	After Stimulation.		
Veins ligated *on both sides*	0·199	0·167 (*10*)*	Fall	Coil, 6 centimetres.
	0·173 (*20*)	0·199 (*10*)	15	—
	0·183 (*15*)	0·241 (*10*)	30	Blood-pressure, 60 millimetres Hg.†
	0·266	0·249 (*8*)	Fall	Marked hyperpnœa throughout experiment. Coil, 6 centimetres.
	0·300 (*23*)	0·255 (*8*)	,,	—
	0·317 (*13*)	0·321 (*5*)	No change	Blood-pressure very low.
	0·177	0·168 (*6*)	Fall	Coil, 6 centimetres.
	0·138 (*12*)	0·110 (*7*)	,,	—
	0·120 (*20*)	0·140 (*7*)	16	Blood-pressure, 60 millimetres Hg. Coil, 4 centimetres.
Veins ligated *on one side*	0·200	0·225 (*8*)	12·5	The right adrenal vein was not ligated.
	—	0·268 (*28*)	—	—
Combined experiment.	0·216	0·238 (*9*)	10	Coil, 6 centimetres. Adrenal veins intact.
	0·202	0·196 (*8*)	Fall	Adrenal veins ligated.

adrenalin. They are, for example, still subject to sympathetic control in preparations that are kept alive outside the body by artificial perfusion. The portion of the nerve path for which the presence of adrenalin in the blood is necessary is probably the "receptive material," which is believed to lie between the

* The figures in italic indicate the number of minutes elapsing between the removal of the various samples of blood. In order, the figures are to be read from left to right.

† Unless otherwise noted, the arterial blood-pressure was between 90 to 120 millimetres Hg.

nerve terminations and the cell substance. The existence of such a receptive material is deduced from experiments by Elliott and others (20), who have shown that the stimulating action of adrenalin on muscle fibre is increased after the nerve fibres have degenerated, or even, in some cases, after they have merely been cut (21). It is believed that the denervation in these cases causes the receptive material to accumulate in the cells, and that this material is directly excited by the presence of adrenalin. In the case of the glycogenic function, however, the receptive material does not appear to accumulate immediately after cutting the nerves, for, if it did so, stimulation of the splanchnic nerves, with the adrenals intact but the hepatic nerves cut, should produce a hyperglycæmia which is at least as marked as that which occurs when these are also intact. We may therefore conclude that it is impossible, by stimulation of the splanchnic nerve, to bring about a sufficient concentration of adrenalin in the blood to excite the receptive substance to anything like the same degree as that which follows stimulation of the nerve fibres, although the slight increase in blood-sugar sometimes observed (see p. 66) may indicate that a moderate degree of adrenalin stimulation has occurred.

It is evident that this view of the rôle of adrenalin is not out of harmony with the fact that an excess of adrenalin in the blood should cause a stimulation of the glycogenolytic process. This effect of adrenalin is no doubt directly on the hepatic nerve terminations, or, rather, on the receptive substance, and not indirectly because of stimulation of the nerve centres, for it follows when the injections are made into a branch of the portal vein after the hepatic plexus has been cut. The following table of results clearly demonstrates this :

THE EFFECT ON THE SUGAR OF THE CAVA BLOOD PRODUCED BY INJECTION OF ADRENALIN INTO THE PORTAL VEIN.

No. of Experiment —Weight.	Amount of Adrenalin Injected.	Per Cent. Reducing Substance, Before.	Per Cent. Reducing Substance, After.	Time Blood Taken after Injection.	Remarks.
69 (14·9 kg.)	12 c.c. (1 to 5,000)	0·257	0·272	at end of injection	Similar injection of Locke's solution = no effect.
	—	—	0·314	3 minutes later	
	—	0·225	0·343	5 ,, ,,	
73	12 c.c. (1 to 5,000,	0·144	0·179	2·5 minutes	Ditto.
	—	0·228	0·263	2·5 ,,	
	—	0·190	0·263	4 ,,	

To sum up, we may state that the sympathetic nerve terminations that control the glycogenic function can be stimulated either by an excess of adrenalin in the blood or by nerve impulses, but that the latter are incapable of acting unless there is a certain concentration of adrenalin in the blood. It is extremely doubtful whether there can ever be so marked an increase in the secretion of adrenalin into the blood as to excite hyperglycogenolysis by itself; all the conditions, such as piqûre, stimulation of the splanchnic nerve, etc., that have this effect act directly through the hepatic nerves, the excitability of these being, however, simultaneously heightened by an increased secretion of adrenalin.

Returning to our main question, "Does the nervous system serve as the medium of control between the sugar content of the systemic blood and the sugar output by the liver?" we have shown very definitely that there are efferent nerve fibres controlling the glycogenoyltic activities of the liver, and that this nerve control is possible only when there is a certain amount of adrenalin in the blood.

But the mere presence of efferent glycogenolytic nerve fibres does not necessarily imply that it is through these that the output of sugar by the liver is ordinarily regulated, for we have yet to show not only that these nerves may be excited reflexly, but also that this reflex excitation can be accomplished by changes in the chemical composition of the blood.

The Reflex Control of Hepatic Glycogenolysis.

Amongst the earliest published researches on the physiology of sugar control in the animal body are several showing (commonly) that stimulation of the central end of the vagus nerve is followed by glycosuria.

Bernard demonstrated in well-fed dogs after stimulation of the vagus the appearance not only of glycosuria, but of hyperglycæmia, and although he called attention to the interference with the respiratory movements which accompanies such stimulation, he concluded that the hyperglycæmia depended on afferent stimulation of the centre in the medulla (the glycogenic centre), from which arise the nerve fibres that control the sugar output of the liver. Eckhard, Külz, and Laffont confirmed Bernard's discovery, the last-mentioned author pointing out, however, that the glycosuria might be due

to the disturbance in respiration rather than to stimulation of the glycogenic centre (22).

Dolley and the writer found it an easy matter to confirm the results of these earlier investigations. Not only stimulation of the central end of the vagus in the dog and rabbit, but also of the cardiac depressor nerve in the latter animal produced, and sometimes within ten minutes, a very evident glycosuria. The glycosuria was, however, never so striking as that observed in piqûre, and since, in most animals, a certain degree of glycosuria not uncommonly results from anæsthesia (see p. 187), it became of importance to collect more data regarding the reducing power of the blood after stimulation of the sensory nerves. This was all the more necessary because it was noted that glycosuria might become established without any increase in the blood-sugar, or conversely, that the urine might remain normal in cases where there was quite a distinct increase in blood-sugar. Under ether alone there is seldom, in our experience, any noteworthy increase in the reducing power of the blood. If a change does occur, it is a slow continuous one, and can but rarely cause any confusion in the interpretation of the results.

Examination of the reducing power of dog's arterial blood, removed at periods varying from thirty-five minutes to two and a quarter hours after the first application of an intermittent stimulus to the central end of one or other vagus nerve, showed a distinct increase over the normal. The stimulus was usually applied at intervals of a few minutes apart, each period of stimulation lasting about two minutes. In seven dogs the average per cent. of reducing substance in the femora blood was 0·169 before stimulation, and 0·222 about an hour after the first application of the stimulus (23). In two of these experiments the urine acquired unusually high reducing powers, 5 per cent. was found in one case forty minutes, and 8 per cent. in another two and a quarter hours after the first application of the stimulus.

Such results would seem at first sight unequivocally to demonstrate the existence in the vagus nerves of afferent fibres acting on the glycogenic centre. Before such a conclusion is warranted, however, another possible cause for the hyperglycæmia must be considered—namely, the interference with the pulmonic ventilation, which almost invariably follows stimulation of the

vagus nerve. The effect on the respiratory movements usually consists in a tetanus, immediately following the application of the stimulus, succeeded by rapid and shallow breathing movements. These effects are usually much more marked early in the experiments than later. They immediately strike an observer as being likely to lead to considerable interference with the proper arterialization of the blood ; as being capable, in other words, of bringing about a condition of partial asphyxia. Now, it is well known that asphyxia, however produced, is almost invariably followed by hyperglycæmia and glycosuria. The exact cause of this we shall defer till later (see p. 177), but meanwhile we must consider the possibility that it is because of this respiratory disturbance, rather than afferent stimulation of the glycogenic centre, that vagal hyperglycæmia result.

The Effect of Stimulation of Central End of Vagus on Blood-Sugar in Dogs not receiving Oxygen.

Time after Start of Stimulation.	Per Cent. Sugar in Blood.	Per Cent. Sugar in Urine.
75 minutes	0·273	Small amount
110 ,,	0·313	1·7
55 ,,	0·171	—
95 ,,	0·231	7·1
145 ,,	0·246	8·0
40 ,,	0·218	Abundant
35 ,,	0·235	,,
60 ,,	0·201	2·9
70 ,,	0·187	Abundant, and diuresis
16 ,,	0·234	Abundant
40 ,,	0·270	5·0 (diuresis)

To test this hypothesis it is necessary to modify the experiments. This we have done in two ways. The first of these consisted in a repetition of the above described experiments, with the difference that the development of an asphyxial condition of the blood was prevented by delivering into the bronchi through a narrow tube (gum-elastic catheter) a continuous stream of washed oxygen. It was first of all shown by Hirsch, and later by Sollmann, that by such oxygen insufflation animals can be kept alive and in good physiological condition, even in the absence of any respiratory movements. Meltzer has shown that air can be

used in place of oxygen. The continuous stream of oxygen or air in the air passages adequately ventilates the alveoli—that is to say, it removes the carbon dioxide from the blood, and adequately supplies this with oxygen.

It was found in dogs so treated that stimulation of the central end of the vagus did not cause any increase in the reducing power of the arterial blood, even in periods exceeding three hours from the beginning of the nerve stimulation. Occasionally, however, there appeared quite a considerable amount of reducing substance in the urine.

THE EFFECT OF STIMULATION OF CENTRAL END OF VAGUS NERVE ON BLOOD-SUGAR IN DOGS, RECEIVING INTRATRACHEAL INSUFFLATIONS OF OXYGEN.

Time after Start of Stimulation.	Per Cent. Sugar in Blood.	Per Cent. Sugar in Urine.
30 minutes	0·176	
75 ,,	0·188	Present
110 ,,	0·178	
57 ,,	0·163	
77 ,,	0·070	Present
107 ,,	0·090	
20 ,,	0·264	Sugar-free
65 ,,	0·297	Present
95 ,,	0·155	Abundant
60 ,,	0·142	0·5
160 ,,	0·173	2·5
180 ,,	0·182	2·0
60 ,,	0·153	
120 ,,	0·157	The mixed urine contained 0·6 per cent.
150 ,,	0·157	
190 ,,	0·166	

Although at first sight these results would seem to indicate that the vagus cannot contain afferent fibres to the glycogenic centre, this conclusion is not inevitable, for it is possible that with over-arterialization of the blood a small increase in the amount of sugar delivered into it would not cause hyperglycæmia because of the excess of sugar being burnt up. It is well known that it is impossible to stimulate increased combustion in the animal body by increasing the oxygen-supply to the tissues, but it may be otherwise when there is excess of dextrose derived from glycogen in the blood. Such dextrose is possibly different biochemically from dextrose as we find it in the chemical laboratory (see p. 84), and it is at least possible that this

glycogen-dextrose, as we may designate it, is capable of oxidation when it is present in the blood and tissues in excessive amount along with an abundance of oxygen. We have not as yet been able to subject the matter to the test of experiment, but have observed that the hyperglycæmia, following stimulation of the great splanchnic nerve, is considerably lessened when the blood is over-arterialized by the above method (see Table on p. 61).

The second method by which we have attempted to show whether there are really afferent fibres to the glycogenic centre in the vagus nerve has consisted in examining the blood directly after its discharge from the hepatic veins at short periods of time after the application of the stimulus to the vagus nerve. If there really are afferent glycogenolytic fibres in this nerve, the effect of their stimulation on the sugar output by the liver should be comparable with that produced by stimulation of the splanchnic nerve itself. It will be remembered that an increase amounting to from 10 to 30 per cent. is produced within a few minutes in the sugar of the cava blood when this nerve is stimulated. By stimulation of the central end of the vagus, however, either *with* or *without* intratracheal insufflation of oxygen, no such changes occur ; the percentage of sugar remains practically constant.

It has been stated by Starkenstein (24) that the reducing power of the aqueous humour becomes increased in adrenalectomized rabbits (one experiment) when the central end of the vagus (left) is very strongly stimulated. Such a result would contradict the conclusion drawn above that nervous control of the glycogenic function is impossible in the absence of adrenalin, and it would serve to demonstrate afferent fibres to the glycogenic centre. One experiment, indefinitely reported, can, however, scarcely be considered adequate support for such conclusions.

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CHAPTER IV

THE RELATIONSHIP OF THE DUCTLESS GLANDS TO SUGAR METABOLISM

When we compare the nature of control of the various glands that participate in digestion, we find that, in those which secrete at short notice, such as the salivary and gastric glands, it is the nervous system that mainly dominates their activities; whereas, as we descend the gastro-intestinal tract, and promptitude in response of the glands comes to be of less importance, the nervous system participates less and less in the control, and in its place regulation is effected by means of substances present in the blood (hormones). These hormones are delivered into the blood by the epithelial cells which line the gastro-intestinal tract, and they are produced by an action on these cells of certain constituents of the semidigested food, derived from processes occurring at an earlier stage in digestion than that for which the secretion of the gland in question is required. Thus, set in action by stimulation through the nervous system, the subsequent activities of the digestive glands appear to be kept up by an automatic stimulation of each succeeding process by the products of an earlier stage. The semidigested products of gastric digestion, for example, by producing the hormone gastrin, stimulate further secretion of gastric juice; the acid secreted in this juice, when it reaches the duodenum, excites the formation of secretin, and the trypsinogen in the pancreatic juice that is secreted in response, again acts on the intestinal glands, and calls forth from them a secretion of entero-kinase.

The glycogenic function of the liver is to be considered as the first step in the assimilation of carbohydrates; the processes of digestion and absorption have been completed, and the sugar is now carried to the liver, where the greater part, if not all, of it is polymerized to glycogen, which may become

stored for a longer or shorter time according to the needs of the organism for carbohydrate.

Is it not possible, then, that it is by hormone control that the output of sugar from the liver to the systemic blood is regulated? Reasoning by analogy, we should suspect this to be the case. This glycogenolytic hormone might be (1) dextrose itself, or (2) substances other than dextrose.

We shall proceed to consider the possibility that the hormone is dextrose.

At first sight it seems perfectly simple to ascribe the control of the sugar output of the liver to the amount of this substance already present in the systemic blood, the stimulus to increased production being, of course, a lessening in the amount in the blood. But there is at least one serious difficulty in accepting such an hypothesis, namely, the fact that the main blood-supply of the liver comes to it through the portal vein, in which, during the process of absorption, there may be an enormous increase in the amount of sugar unaccompanied by any very constant change in the amount of sugar in the systemic blood. Sometimes there is a slight rise, at other times a fall in this (see p. 54). In other words, there may be quite a marked *hypo*glycæmia in the systemic blood, which, by the time that it gains the liver cells, becomes changed to a *hyper*glycæmia because of the sugar added to the blood in its passage through the walls of the intestines. The case is not so simple, therefore, as at first sight it may appear to be.

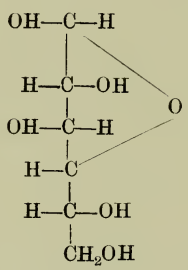
On the strength of the above observation, however, we must not finally dismiss the hypothesis, for it is possible that it is only *one variety* of dextrose in blood which is capable of controlling the glycogenolytic process.

The existence of two distinct varieties of dextrose (α and β) was first of all indicated by Emil Fischer, who, by dissolving dextrose in methylic alcohol in the presence of dry hydrochloric acid, found that the reducing power of the solution gradually disappeared, and after removal of the hydrochloric acid by means of lead and evaporation of the solution there resulted the crystallization of a compound of methyl and glucose called α-methyl-glucoside. The isomeric compound β-methyl-glucoside was later separated from the mother liquid. These glucosides differ from one another not only in several of their chemical reactions, for example, in their melt-points, but also with regard

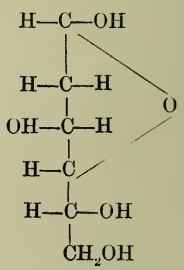
to their behaviour in the presence of enzymes ; the *a* variety is decomposed into methyl alcohol and glucose by maltase—an enzyme present in yeast—whereas this enzyme has no action on the *β* variety, which, however, is decomposed by emulsin—an enzyme present in bitter almonds (E. F. Armstrong, 1).

These methyl-glucosides are analogous with the disaccharides, for in these one glucose molecule is linked with the other in the same way that the methyl group is linked with glucose in methyl-glucoside. Thus maltose is glucose-*a*-glucoside, and isomaltose is glucose-*β*-glucoside. The former can be hydrolyzed by maltase only, and the latter by emulsin only.

The relationship of these compounds is shown in the following formulæ, in which dextrose is represented, not as an open chain, as in the older formulæ, but as a partly closed chain, one oxygen atom serving as the link which joins the carbon atom, represented in the open chain formula as the one to which the aldehyde group is attached, with the fourth carbon atom in the chain :



a-glucose.



β-glucose.

In the methyl glucosides the methyl radicle takes the place of the H atom in the hydroxyl group attached to the first carbon atom. In maltose this same H atom in *a*-glucose is displaced by a second molecule of *a*-glucose. In isomaltose, a *β*-glucose molecule displaces it in the *β*-glucose molecule.

Now, it is possible that glycogen is built up out of glucose molecules that are all of one variety, so that when it becomes hydrolyzed by the action of glycogenase—the enzyme that produces dextrose from glycogen—the sugar that passes into the systemic blood is not the equilibrated mixture of *a*- and *β*-glucoses which is absorbed from the intestine, but only that particular variety of glucose of which the glycogen is composed.

In such a case it might be supposed that the rate of hepatic glycogenolysis is dependent solely upon the concentration in the blood of that variety of dextrose which is produced by it. A certain amount of this same variety of dextrose will, of course, be absorbed from the intestine, and be added to that already present in the portal blood; but the other variety, simultaneously absorbed, will be without influence in the control of the glycogenolytic process.

To test this hypothesis, it would be of interest to compare the effect produced on the discharge of sugar from the liver by injection into the portal vein of ordinary dextrose, and of an equal amount of dextrose produced by the enzymic hydrolysis of glycogen. As having a bearing on this same question, it would also be of value to know whether the organism can more readily destroy such glycogen-dextrose than ordinary dextrose.

But dextrose itself may not be the hormone which controls the glycogenolytic process; indeed, it is well known that there are many other constituents of the blood which can influence this process. These may be divided into three groups:

1. Secretions of ductless glands, such as the adrenals, the isles of Langerhans, and the parathyroids. The supposed internal secretions of the thyroid and hypophysis must also be considered.

2. Products of the metabolism of dextrose, such as carbonic acid, lactic acid, and the so-called leucomaines of Lepine.

3. The inorganic salts in the blood.

In the following pages we proceed to a review of the work bearing on the possible relationships of the ductless glands to the amount of sugar in the systemic blood. In doing so we make the assumption that the hormones, which these glands probably produce, owe their influence, if any, on the sugar content of the blood, to their effect on the glycogenic function of the liver rather than to one exercised on the destruction of dextrose in the tissues. This view is contrary to that taken by many recent investigators, but it is probable that in the present state of our knowledge it is the safest one to adopt. Reasons for this course have already been given in connection with the relationship of the adrenal gland to the nerve control of the glycogenolytic process. Others will be given when we come to consider the influence of the

pancreas. We shall defer till later the influence which the reaction and saline composition of the blood has on the glycogenolytic process.

The Influence of the Internal Secretion of the Adrenal Gland.

Besides what has already been considered in connection with this subject, the following observations are of importance :

It has been known for over ten years that excess of the active principle of the adrenal gland in the blood causes glycosuria (2).

The glycosuria is accompanied by hyperglycæmia, and it does not matter whether a saline extract of the fresh gland be employed or one of the pure products, such as the adrenalin chloride of Aldrich and Takamine, the epinephrin of Abel, adrenin, or the synthetic product.* Although more marked in animals that contain large reserves of glycogen, adrenalin can still cause glycosuria and hyperglycæmia when given to starved animals. It has, however, been stated that a smaller dose suffices to establish the condition when there is an abundance of glycogen than when this is scanty in amount or absent altogether (Ritzmann, 3). A most significant fact is that when adrenalin is repeatedly given in increasing doses to rabbits, whose tissues have been deprived of all traces of glycogen by starvation followed by the administration of strychnine, it causes large quantities of new glycogen to become deposited in the liver, although the muscles remain glycogen-free (Pollak, 4). It may also cause hyperglycæmia and glycosuria after the glycogen has been cleared out by means of phloridzin (5). The internal secretion of the adrenal gland must therefore be of importance, not only in the control which it can exercise on the production of sugar out of glycogen, but also in its control over the production of sugar out of proteins, a process which, as we shall see later, supervenes in the diabetic animal, when all available glycogen has been used up.

The glycosuria produced by adrenalin lasts just so long as any excess of this substance remains in the blood, and it is more or less proportional in intensity to the rate of administration of

* For convenience, we shall use the word *adrenalin* to mean any of these preparations, unless when it is necessary to be more specific, when the exact name of the product used will be employed.

the drug. The hyperglycæmia and glycosuria produced by a given amount of adrenalin are more marked when the subcutaneous, rather than the intravenous method, of administration is employed. In an observation on a rabbit, the intravenous administration of 2 milligrammes of adrenalin did not cause any glycosuria, whereas 1 milligramme given subcutaneously was followed by a considerable degree of this (Pollak, 7). Similarly, intramuscular injection is less effective than subcutaneous.

Various observers have found that the repeated administration of adrenalin brings about a certain immunity, in the sense that further injections do not any longer produce glycosuria. This result does not, however, appear to be because the adrenalin fails to act on sugar production, but because of a change in the permeability of the kidney (4, 6). The threshold of permeability becomes less, so that, although the percentage of sugar in the blood is rising, less of it is appearing in the urine.

The effect of excision of the glands, or of their disease, on the control which the nervous system exercises over hepatic glycogenolysis, has been discussed elsewhere (see p. 64).

The Relationship of the Pancreas to Sugar Metabolism.

In no other of the many causes of diabetes has greater interest been shown than in that due to disturbance in the pancreatic function. Many of the earlier clinicians, who followed cases of diabetes mellitus into the post-mortem room, noted that definite morbid changes in the pancreas were a frequent accompaniment of the disease. Prompted by these observations, several investigators attempted experimental extirpation of the gland, but did not succeed in producing glycosuria in the few animals that survived the operation. Their failure, no doubt, resulted from incomplete extirpation. To reduce the severity of the operation, Claude Bernard injected oil into the pancreatic duct, and tied it; but he succeeded in keeping only two dogs alive for any length of time, and these did not exhibit glycosuria. Nor were other investigators, who adopted similar methods, any more successful. It looked as if the pancreas had very little to do with the cause of diabetes. In the year 1889 Minkowski and v. Mering in Germany, and

de Dominicis in Italy, by thorough extirpation of the gland, succeeded in producing in dogs a marked and persistent glycosuria, accompanied by many of the other symptoms of diabetes. The first two authors attributed the condition to removal of an internal secretion.

Very important observations were made some years later (in 1895) by Sandmeyer (9), who found that the partial removal of the pancreas in dogs caused at first a temporary glycosuria, which afterwards became more and more marked, until the condition typical of complete depancreation developed. In some of the animals no glycosuria developed at first, but did so later on. The importance of these investigations lies in the fact that they furnish us with an experimental diabetes which, in its course, is remarkably like that observed in the clinic. It would be of little value to review in any detail the investigations which followed this discovery, for there are already in existence not a few monographs in which practically every piece of work bearing on the subject has been thoroughly and critically considered (8).

The aspect of the question which it is necessary for us to examine at present concerns not so much the effects of the removal of the gland on the general physiological condition of the animal, as the evidence which such researches affords regarding the manner in which the pancreas is associated with perversions of carbohydrate metabolism. We are endeavouring to discover the nature of the processes which control the output of sugar by the liver, and its subsequent behaviour in the tissues, and, for the present, we are not concerned in the effect on the organism which the accumulation of sugar entails.

There are in general two questions to be considered : First, why pancreatectomy disturbs carbohydrate metabolism ; and, secondly, whether this disturbance involves the glycogenic or the glycolytic mechanism. Regarding the nature of the association between the pancreas and carbohydrate metabolism, several possibilities exist : (1) That the external secretion—*i.e.*, the secretion into the intestine—is essential for the proper digestion and assimilation of carbohydrate ; (2) that the extirpation of the gland seriously damages the underlying nerves and sets up a constant irritation of the hepatic branches, and therefore a constant production of dextrose by the liver ; (3) that the gland has the power of neutralizing or destroying certain

toxic substances in the blood while this is circulating through it, and that these toxic substances interfere with the glycogenic or glycolytic functions; (4) that the gland produces an internal secretion—*i.e.*, secretes some substance into the blood which exercises "hormone" control over the glycogenic or glycolytic functions, or which destroys toxic substances that would otherwise affect these processes.

There is one well-known experiment, the results of which, once and for all, precludes the first-mentioned possibility. When the "processus uncinatus" (a part of the vertical portion of the pancreas that is not, like the rest, intimately applied to the duodenum, but is separated from it by a mesentery) is stitched into the subcutaneous tissues of the abdominal wall, and the remainder of the pancreas then removed, no diabetes follows (Minkowski, Hédon, 11). Under such conditions no secretion of pancreatic juice is possible, for this graft sends no duct to the duodenum, and yet there is no evident disturbance in carbohydrate metabolism. The digestion of food in the intestine is, of course, more or less disturbed by removal of the pancreatic juice, and fat absorption is materially interfered with (Lombroso, 14), but this faulty digestion has nothing to do with the occurrence of glycosuria. On the contrary, when raw pancreas is given by mouth to depancreated animals, a marked *increase* in in the glycosuria has been observed to occur. This is not the case when cooked pancreas is given, and the result is no doubt due to the better utilization of food (Sandmeyer, 15). The results of the above "graft" experiment furnish also the strongest argument against the view that irritation of the solar plexus is responsible for the hyperglycogenolysis, for there must be just as much irritation of this structure in such an operation as when the whole gland is exsected; and, moreover, when the graft itself is subsequently removed—an operation which cannot disturb the plexus—glycosuria, etc., of the usual intensity immediately sets in. In spite of such apparently overwhelming proof, Pflüger for long maintained that the possibility of reflex nervous stimulation of the glycogenolytic function, as a result of these operations, was not excluded. Having found that a certain degree of glycosuria follows removal of the duodenum in frogs, although this is not the case in dogs (16), this author thought that nerve impulses which inhibit the glycogenolytic process must be transmitted to the liver from the pancreas by

way of the nerves running between the pancreas and duodenum. In the above-described "graft" experiment, according to this authority, some of these fibres would remain intact, and no diabetes would develop until after the graft had been removed, when, the inhibiting impulses being absent, glycogenolysis would be stimulated on account of impulses transmitted from the glycogenic centre.

To Hédon, and later to Lombroso and W. G. Macallum (17), is due the credit of finally establishing the fact that it is to the absence of some influence of the pancreas itself, independently of any nerve connections, that the diabetes which follows its extirpation is due. Lombroso's experiment was briefly as follows: In a female dog the processus uncinatus was grafted in the abdominal wall, and a fistula established to drain its secretion. The remainder of the pancreas was then resected, with the result that a mild glycosuria (0·3 per cent. dextrose) followed. A month later the pedicle connecting the graft with the duodenum was ligated and cut, and, although for a period of four days a more decided glycosuria (1·5 per cent. dextrose) occurred, it then cleared up, indicating therefore that the nerve connection was not the essential influence that had hitherto prevented it. Eight days later the graft was removed, and intense and quickly fatal diabetes followed. In the month during which the graft had been connected with the duodenum by a pedicle, a new arterial blood-supply had become established between it and some of the intramuscular arteries, so that, after the pedicle was cut, the graft could still exercise its control through the blood. In Macallum's experiment, which will be described in another connection, equally convincing proof of the "internal" influence of the pancreas is offered (17).

We are now left with two possible explanations for the influence of the pancreas—an internal secretion, and a local action taking place in the pancreas itself on toxic substances carried to it in the blood. As to which of these is the true explanation, the final verdict cannot yet be pronounced. Against the view that there is an internal secretion, is often advanced the fact that we cannot abolish the glycosuria in depancreated animals by injecting extracts of pancreas. In myxœdema the symptoms are said to be due to a diminished internal secretion of the thyroid gland, the crucial test for this belief being the fact that administration of thyroid extract immediately clears up the con-

dition. The analogy is, however, not well drawn, for in a pancreatic extract, besides any substance which may have an action on metabolism, are also several very powerful ferments which may quickly destroy that substance ; and, moreover, there may be no ready-made internal secretion in the pancreatic cells themselves: it may be produced in small quantities at a time, according as it is required, or it may exist in the gland as a precursor, and become activated only after it has been secreted into the blood.

There are certain recent observations that are usually considered as evidenced in favour of the "hormone" hypothesis, and which it is important that we should examine with a view to seeing in how far they really do support this view. These are by Forschbach, Carlson and Drennan, and Knowlton and Starling.

Forschbach (18) succeeded in four experiments in bringing about a union between two dogs of similar size and age by sewing skin, muscles, and peritoneum together (parabiosis). After the wounds had healed, blood and lymph came to circulate through newly formed vessels established between the two animals, so that when iodine was administered to the one, it appeared in the urine of both. When the pancreas was now removed from one of the dogs of each pair, it was found that this animal did not develop the usual degree of glycosuria—indeed, in two of the experiments remained a-glycosuric for from thirty-six to forty hours after the operation. The wound suppuration and depressed general condition, which are usually so marked after pancreatectomy, were also of slight degree. In two of the experiments the normal dog developed slight glycosuria, as a result of the pancreatectomy of its partner, and this happened in one case although the depancreated animal was a-glycosuric. After separation of the dogs, in one experiment, the glycosuria in the depancreated animal seemed to become more marked.

Ingenious and important as experiments of this type must be, the results of the above observations of Forschbach are too uncertain and confusing to permit of any conclusion being drawn from them. There are really three explanations possible for the results of this observer : (1) That the pancreas of the normal animal supplied sufficient hormone to control the sugar metabolism of both animals ; (2) that toxic substances, produced in the depancreated animal, were destroyed in the pancreas of the normal animal ; (3) that the excess of sugar in the blood of the depancreated animal became utilized by the normal animal.

The fact that in two of the experiments the normal dog became glycosuric could be explained in any of these ways, for it may be considered that the essential change produced by the parabiosis is that the pancreas of one animal has now to supply hormone, or destroy a toxin, for two. That there was a *free* blood circulation, at least in one of the pairs, was shown by finding that lactose injected in the one animal appeared to about an equal extent in the urine of both. It is plain, therefore, that the same condition as that produced in the parabiosis experiments becomes established when half the pancreas is removed from one animal, so that the only really important interest of those experiments is that they indicate that the mechanism which is disturbed by removal of the pancreas has nothing to do with the nervous system.

Carlson and Drennan (19) have supplemented these observations by finding that pancreatectomy in pregnant dogs, near full term, is not followed by the usual degree of glycosuria ; indeed, in some cases, is followed by no glycosuria whatsoever. These observers believe that "the internal secretion of the fœtal pancreas passes through the uterine membranes in sufficient quantity to prevent diabetes in the mother." It is unfortunate that the behaviour of the blood-sugar was not observed in these experiments, and that more evidence is not offered of the thoroughness of the pancreatectomy.

The same authors have also found that the amount of sugar excreted by a depancreated dog in twenty-four hours becomes less after the intravenous injection of blood from a normal animal. They have shown that the mere dilution of the blood is not responsible for the diminution in sugar excretion ; but when we remember the great susceptibility of the excretory function of the kidney towards dextrose (see p. 46), it is again unfortunate that the blood-sugar was not examined.

Taking the observations as a whole, it seems justifiable to conclude that the pancreas owes its influence over carbohydrate metabolism to an internal secretion rather than to any power which its cells might have of removing or neutralizing toxic substances while the blood is circulating through it. This conclusion is greatly strengthened by the experiments of Knowlton and Starling, in which observations were made on the rate at which sugar disappeared from blood that was repeatedly perfused through an isolated mammalian heart prepara-

tion (dog). They found that the consumption of dextrose per hour and per gramme heart muscle was usually less than 1 milligramme when the blood and heart were those of a depancreated dog, against 4 milligrammes when the heart and blood were from a normal animal. When the heart was from a diabetic animal, the blood from one that was normal, the dextrose consumption for the first hour was still low, but it increased steadily during the next two hours, and, conversely, when diabetic blood was perfused through a normal heart, the consumption, although almost normal during the first hour, fell off markedly during the second and third.

These results are most simply interpreted as indicating that some hormone necessary for the direct utilization of dextrose by the tissues is absent in the diabetic blood. This view is further supported by the observation that the addition of a boiled protein-free extract of pancreas, made with faintly acid Ringer's solution, to the blood of a diabetic dog, restores almost to normal the glycolytic power, as observed in the above manner. If these observations can be confirmed for the skeletal muscles, they will constitute the most important contribution that has recently been made in the physiology of the carbohydrates (20).

Whichever view may be the correct one, there can be no doubt that the pancreas exercises some very important control over carbohydrate metabolism, and it remains for us to inquire as to the exact anatomical structure of the gland that may be responsible for this influence. Two perfectly distinct types of secreting cells are present in the gland, the one composing the secreting acini, the other collected into irregularly distributed groups, which are more or less oval in shape, and which vary in size in different species. These are known as the "isles of Langerhans," and, on account of their abundant blood and nerve supply, and the fact that the cells which compose them stain indifferently with any kind of stain, while those composing the acini stain readily, many anatomists have considered them as quite independent of the acinar cells. Others believe, however, that the one kind of cell may change into the other; for example, that the insular cells are nothing more than completely exhausted acinar cells. When secretin is repeatedly injected, so as to exhaust the gland, it has been found by Dale that the acinar cells lose their power to stain, and come to be

indistinguishable from the cells of the islets. The islets therefore look as if they were incorporating the acini with themselves, and the whole picture gives the impression that the islet cells are merely stages in the life-history of the secreting. This interpretation is not, however, generally accepted by those who have repeated Dale's experiments (21).

Transitional forms of cells have also been described. Thus, when most of the pancreas is removed, and the remainder is grafted, in the spleen, for example, out-growths develop from the ducts, and the cells of these may afterwards become, either acinar or insular (Kyrle, 22). Connections between the isles and the ducts of the secreting acini have been described in the pancreas of man, so that the possibility exists that the islet cells produce some of the enzymes of the pancreatic juice (Laguesse).

The pathological evidence has usually been considered of great weight in support of the view that diabetes mellitus is due to disease of the pancreas. Undoubtedly, in many cases of this disease, a pathological condition of the pancreas is found in the pancreas after death, but this fact, taken by itself, does not warrant the conclusion that the pancreatic lesion has been the *cause* of the *disease ;* it may be merely an effect of it, just as are cataract, gangrene, etc.

Not infrequently, too, even the most painstaking microscopical examination of the pancreas from cases of typical diabetes mellitus fails to reveal any abnormal condition whatsoever, either in the acinar or the insular cells. But for this reason alone we cannot discard the view that the principal cause of the diabetes has been failure of an internal pancreatic secretion, for it is quite possible that there may be derangement of function, unaccompanied by any demonstrable anatomical change.

On the other hand, glycosuria not infrequently is absent in cases in which there is found to be a practically complete destruction of the pancreas, as in necrosis and hæmorrhagic pancreatitis. In these acute cases of pancreatic destruction, however, it may well be that death has so quickly followed the dissolution of the gland that the want of its influence on carbohydrate metabolism has had no time to develop. It is interesting in this connection to note that experimental necrosis of the gland in dogs, produced by injecting oil in the ducts, fails also to cause glycosuria.

Although the pathological evidence cannot therefore help us

very much in deciding as to the relative importance of pancreatic disease as a cause of diabetes, yet in those cases in which there *are* lesions of the pancreas—for undoubtedly such occur in most cases—we have an opportunity of seeing which type of gland cell it is that exhibits the pathological change.

Notwithstanding the immense amount of investigation that has been devoted to this question by pathologists (*cf.* 23), there is great diversity of opinion as to the conclusions which should be drawn. After a comprehensive review of the literature, Lombroso (14) concludes that the pancreatic lesions, when they exist, may be either in the acini or in the islets, or that both structures may be involved to an approximately equal degree. The islets, too, may be extensively diseased in cases that are not diabetic.

It has been attempted to throw further light on this question by experiments in which the pancreas is made to degenerate, either by ligation of the ducts or by injecting them with oil or paraffin. After ligation of the ducts, no diabetes, as a rule, develops, although a marked atrophy affects the pancreas. This atrophy involves both acinar and insular cells; a certain number of both kinds of cells may remain normal. When the atrophy is extensive, diabetes supervenes. There is, however, much uncertainty as to whether the occurrence of this depends on the disappearance of one variety of cell, and this uncertainty appears to be due to confusion of persistent or regenerated acini with islets, for in interpreting the changes produced by such experiments, the possibility must be kept in mind that all of the ducts may not have been ligated,or injected, and that the persistent acini or islets are connected with these. Much more convincing, therefore, are the experiments in which the graft of pancreas is completely isolated from the main gland before the latter is removed. Such experiments, in which particular attention was paid to the histological changes, have been recorded by Lombroso (8) and W. G. Macallum (17). The former observer has insisted that all of the acini do not degenerate nor do new ducts become developed, and that the physiological effect on the animal (dog) is definitely proportional to the degree of degeneration of the graft. This applies to acini as well as to islets. When rabbits instead of dogs were employed, the results were less definite, probably because of the peculiar spread-out arrangement of the pancreas in this animal. Macallum

isolated a portion of the pancreas from the main gland, but left it in its mesentery, and ligated its ducts. After some time the isolated portion had atrophied until it appeared as a mere thickening of the mesentery. Excision of the main gland was now performed, but the animal remained a-glycosuric ; indeed, could assimilate 40 grammes of dextrose. Subsequent removal of the graft, however, immediately caused diabetes, and histological examination of the graft showed, besides remnants of pancreatic ducts, what appeared to be enlarged isles of Langerhans. These may, however, have been merely modified acini. The histological differentiation between acini and islets is not sharp enough, especially when both are more or less modified by atrophy, to make it possible to decide which is present.

Taking the results of these experiments as a whole, it may safely be concluded that they offer no justification for the view that the " internal secretion " of the pancreas is derived solely from the isles ; the acini may also be its source.

Attempts have been made to bring about increased or decreased production of the internal secretion of the pancreas by the administration of drugs, etc. Such experiments are, however, always open to criticism on account of the uncertainty as to how exactly the administered substance acts. Nevertheless, some interesting observations are on record ; for example, that an exclusive carbohydrate diet causes hyperplasia of the isles. It can be supposed that under such conditions—of excess of sugar in the blood—a stimulation of the internal secretion of the pancreas might occur. But the results are very contradictory, for another author found that when two-thirds of the pancreas was removed, and, besides the carbohydrate feeding, sugar was injected intravenously, the isles became smaller, and the cell granules diminished in number ; furthermore, injection of other substances than sugar produces the same changes.

Quite apart from the uncertainty of the results, it is difficult to see how these drug experiments are to help us in deciding as to the rôle of the pancreas in the pathogenesis of diabetes.

There are two stages in the metabolism of sugar over which the internal secretion of the pancreas may exercise control ; these are the glycogenic and the glycolytic. There is the possibility that it may be the glycogenic function which is primarily affected by removal of the pancreas, and in such a way as to cause

dextrose to accumulate in the blood, but we must in this case assume that the conversion of monosaccharides into glycogen is a necessary preliminary stage to their ultimate utilization by the tissues. The manner in which this could occur has already been explained, and it remains for us to see whether there is any evidence that the glycogenic function becomes upset by pancreatectomy. The following observations afford this evidence: Minkowski and others have noted that there is no glycogen, or only a trace, in the liver or muscles in depancreated dogs, even after feeding with large amounts of dextrose or starch. Some is present when lævulose is fed (12), which in part explains why in certain cases of diabetes mellitus this sugar should be better tolerated than dextrose. It is significant, too, that the muscles retain their glycogen more tenaciously than the liver.

In frogs, pancreatectomy causes glycosuria, which does not develop when the liver is also removed, and in herbivorous birds, although pancreatectomy is not followed by diabetes, the liver loses the power of forming glycogen when dextrose is fed (v. Noorden, 11). Ligation of the bloodvessels of the liver is also said to cause the glycosuria to disappear in depancreated dogs. For reasons given elsewhere, however (p. 180), too much weight should not be placed on this experiment.

Changes are also produced by removal of the pancreas in the amount of glycogenolytic enzyme present in the blood. We will defer a consideration of these until after we have studied the behaviour of the enzymes (see p. 156); meanwhile, the fact that such changes exist indicates an association between glycogenolysis and the pancreas. On the other hand, it appears that the influence of the pancreas cannot be essential to the building up of glycogen in the liver, for Nishi (11A) has observed that this organ can form glycogen, when it is perfused with Ringer's solution containing dextrose, in turtles from which the pancreas has been removed.

There is therefore no doubt that the glycogenic function is upset by removal of the pancreas, but this does not necessarily mean that interference with the glycogenic function is the only cause of the diabetes. Indeed, as we ordinarily understand this function, such cannot be the case, because the diabetes persists when all carbohydrate food is withheld, the sugar being derived under these conditions from protein.

This new production of dextrose (glyconeogenesis) may occur

because of the need on the part of the tissues for this sugar, and this need may depend on the fact that their glycolytic powers have become so depressed that they can consume a sufficiency of it only when there is a high concentration of dextrose in the blood. According to this view, therefore, the primary fault in pancreatic diabetes would lie in the upsetting of the glycolytic powers, and the glycogenic function would be secondarily involved. The majority of observers have adopted this view. All attempts to demonstrate that, by itself, the blood of a de-pancreated dog possesses less glycolytic power than that of a normal animal have, however, proved futile ; indeed, diabetic blood may actually bring about a higher degree of glycolysis than normal (Knowlton and Starling). On the other hand, as we have seen, when the glycolysis is observed occurring in blood that is made to circulate repeatedly through an isolated heart preparation, a distinct depression is evident in diabetic as compared with normal blood.

The Relationship between the Adrenal Glands and the Pancreas in the Control of Carbohydrate Metabolism.

The pancreas and the adrenal glands evidently influence sugar metabolism in an opposite sense, for the former keeps down the sugar concentration in the blood, while the latter tends to make it rise. It has not unnaturally been supposed that these two influences are constantly at work in the body, so that the sugar concentration at any moment is the algebraic sum of their activities. The advantage of such a reciprocal mechanism of control would consist in the greater promptitude with which a change in the sugar concentration in the blood could be brought about. By suppression of the one and an increase of the other, for example, a greater effect could become developed in a given time than if one alone were to change. According to such a view, the function of the pancreas, in so far as its control over sugar metabolism is concerned, would be to neutralize the action of adrenalin so that, after pancreatectomy, hyperglycæmia supervenes simply because the internal secretion of the adrenal is now permitted, without any check, to act as a stimulus on the sugar output of the liver (Zuelzer, 25). The following experimental evidence has been advanced in favour of this hypothesis :

1. After ligation of the adrenal veins, or extirpation of the adrenal glands, removal of the pancreas (in dogs) causes only slight glycosuria, or none at all.

2. Simultaneous injection of pancreatic extract, and of such amounts of adrenalin as would alone lead to glycosuria, is not followed by this condition.

But although the facts upon which this supposed evidence is based are correct, they are unquestionably to be explained in an entirely different way. The absence or insignificance of glycosuria, following simultaneous excision of the pancreas and adrenal glands, cannot be considered as any proof that the glycosuria, which otherwise follows pancreatectomy, is due to an uncontrolled action of adrenalin, for, after this operation, the dogs are in such a collapsed condition that very few of the functions of the body can be in anything like working order. For one thing, the blood-pressure must be very low, which alone will make glycosuria impossible. We have seen, too, that the presence of some adrenalin in the blood is necessary for the nervous control of the glycogenolytic process.

To diminish this condition of collapse which results from the simultaneous removal of both glands, Mayer and Frouin have performed the operation in stages. Mayer (26) succeeded in keeping three out of eleven recently depancreated dogs alive for an hour after destruction of the adrenal glands by cauterization. In two of them a decrease in the blood-sugar was found just before death, but in the third there was no such decrease. In six cats this author noted a distinct diminution in the amount of urine excreted, and in its percentage of sugar, following removal of the left adrenal gland, the pancreas and the right adrenal having been removed in stages some time previously.

Frouin (26A) allowed longer intervals of time between the operations, and did not remove both adrenal glands, but left one-third of one adrenal in the animal. One dog lived for sixteen, another for twenty days, and it was noted that the glycosuria and polyuria were much less marked than is usually the case in depancreated animals.

Even with these painstaking precautions nothing of value can, however, be concluded from the results.

At first sight the other experimental proof may appear to be unassailable, especially since the observations upon which it depends have been amply confirmed by subsequent investigators.

Indeed, not only extracts of pancreas, but pancreatic juice, and even lymph from the thoracic duct, have been found, when injected simultaneously with adrenalin, to inhibit the glycosuria. The thoracic duct lymph is supposed to convey the internal secretion of the pancreas to the general circulation, further evidence for this being seen in the fact that ligation of the thoracic duct, or the establishment of a thoracic fistula, causes glycosuria (Biedl, 27). A serious source of fallacy in all these experiments has, however, been brought to light by v. Fürth and Schwarz (28). These authors were able to confirm Zuelzer's initial observation that injection of finely disseminated pancreatic tissue rendered adrenalin incapable of producing glycosuria. They even found that the same action was exhibited when protein-free extracts were employed, or when, by gradually increasing preliminary inoculations of pancreas, the animals (dogs) had been rendered immune to the toxic effects of such injections (28A). But exactly the same inhibiting effect on adrenalin glycosuria was obtained when any irritating substance, such as hirudin, colloidal lymphagogues, turpentine, or aleurone suspension was injected intraperitoneally. It seemed possible, therefore, that the peritoneal irritation, which the injection of pancreatic extracts sets up, might depress the excretory function of the kidney towards sugar, and that it was not at all because of an inhibition of the adrenalin influence on sugar production in the liver that the above-described results with such extracts were due.

We have already seen that the excretion of sugar is influenced by the rate of urine formation (p. 46). Bearing this in mind, Fürth and Schwarz studied the influence of such irritation, not only on the amounts of urine, but also on its nitrogen and sodium chloride content. They found the irritation to have little influence on the amount of urine, but, on the other hand, to cause a marked decrease in the excretion of sodium chloride and nitrogen. There can be little doubt that sugar is subject to the same conditions of excretion by the kidneys as those governing sodium chloride and nitrogen. Estimations of the percentage-reducing power of the blood before and after administration of adrenalin to a dog that had been injected intraperitoneally with pancreas showed as great an increase, as a result of adrenalin, as was the case without any pancreas treatment. It is therefore unnecessary to assume any other effect

of the intraperitoneal injections of pancreas than that which is due to irritation, and the consequent decrease in the excretory function of the kidney toward sugar.

To sum up, there is not the slightest experimental evidence that the hyperglycæmia, etc., following pancreatectomy is due solely to an uncontrolled action of the internal secretion of the adrenal gland.

The Relationship of the Thyroid and Parathyroid Glands to Carbohydrate Metabolism.

Considerable interest has recently been taken in certain disturbances in carbohydrate metabolism that are produced by operations on the thyroid and parathyroid glands.

In considering the influence which these glands may possibly exert in this direction, it must never be lost sight of that many other functions are likewise associated with them. The disturbances produced by the removal of the parathyroid glands, for example, include, besides a tendency towards glycosuria, certain nervous symptoms (tetany) that are very characteristic, and are immediately removed by the administration of calcium. The relationship of the thyroid gland to protein metabolism must also be kept in mind.

In the earlier observations of Hirsch, and later of Underhill and Closson, it was shown that removal of both the thyroid and the parathyroid glands in dogs caused a marked lowering of tolerance towards dextrose, given by mouth or injected subcutaneously (29). It was necessary to determine with which of the two glands this influence is associated, for, although some observers hold the view that the parathyroid glands are undeveloped thyroids—that is to say, that under certain conditions, as when the thyroids have been removed, they may develop in the direction of thyroid tissue (Swale Vincent)—yet there is abundance of evidence to show that the two glands possess different and separate functions. This independence of the two glands is perhaps best of all illustrated in their relationship towards carbohydrate metabolism. When the thyroids are removed without injury to more than two of the four parathyroids, the assimilation limit of dextrose is not lowered, but such a lowering immediately becomes evident when more than two of the parathyroids are removed (30). Removal of three

parathyroids along with one thyroid produces just the same change in the assimilation limit as that which occurs when the three parathyroids are alone removed (31). Such results seem to indicate that it is the parathyroids alone that bear a relationship towards carbohydrate metabolism. Some investigators have thought, however, that the thyroids can also exercise a certain amount of control which is opposite in nature to that of the parathyroids—*i.e.*, that it raises the tolerance for sugar—and, on account of the ready acceptance with which this view has been received, it will be necessary for us to consider it a little more closely.

It is based partly on experimental and partly on clinical evidence. The experimental evidence has consisted in observing the effect of thyroidectomy or parathyroidectomy on the glycosurias produced by adrenalin, by ether, or by intravenous injections of dextrose. It was stated that after thyroidectomy in dogs, a given dose of adrenalin caused a less degree of glycosuria than usual, but that the opposite was the case (*i.e.*, the adrenalin more readily produced glycosuria) when more than two of the parathyroids were removed. The administration of thyroid extract to thyroidectomized animals was said to have made them again susceptible to adrenalin. Assuming that the intensity of glycosuria is proportional to the amount of adrenalin injected, it was concluded that the thyroid must decrease and the parathyroids raise the power of the intact animal to utilize sugar (Falta, Rudinger, etc.). These observations on dogs could not, however, be confirmed by Pick and Pineles on rabbits (32). The glycosurias produced by ether and dextrose were said to be influenced by the above operations in the same way as that produced by adrenalin (33).

The clinical observations refer to the behaviour of the assimilation limit towards dextrose in Basedow's disease, in which the thyroid is supposed to be hyperactive, and in myxœdema, in which its function is depressed. In the former disease the limit is distinctly lowered, so that spontaneous glycosuria is quite common, whereas in myxœdema it is said to be raised (34, 35).

There are, however, at least two serious uncertainties with regard to the above evidence. The first of these has to do with the surgical difficulty of removing the thyroids without damaging more than two of the parathyroids. There are, in all, four

parathyroids, two on each side, but their positions with regard to the thyroids may vary considerably. In the herbivora, for example, the external parathyroids are usually so far free from the thyroid that it is easy to excise the one gland without disturbing the other. In the carnivora, however, the separation is less complete, and it is often a most difficult matter to perform a resection of the thyroid without damaging more than two of the parathyroids. It is easy to see that unless extreme care were taken, more than two parathyroids might be accidentally injured in removing the thyroids in dogs; but that this danger would be much less in the case of the rabbit. It is important also to bear in mind that an uncertain number of accessory thyroids may exist, and that these may extend down into the thorax and be incapable of excision.

The other source of experimental error involved in these experiments is with regard to the use that was made of adrenalin glycosuria as an indicator of the state of carbohydrate metabolism, for it has been shown that even in the same normal animal (dog) the intensity of the glycosuria produced by injection of a given amount of adrenalin is not the same at different times. This is true even when all precautions are taken so to regulate the diet that the glycogen content of the liver and other tissues may be as nearly constant as possible. Thus Underhill (36) found that the administration of 1 milligramme adrenalin per kilo body weight to a dog caused a total of 9·7 grammes of sugar to appear in the urine in twenty-four hours. Some time later the same animal, being meanwhile carefully dieted so that it remained practically constant in weight, excreted, after the same dose of adrenalin, only 1·20 grammes of sugar. This is only one of several results, and it once and for all indicates the utter futility of attempting, from the degree of glycosuria produced by injection of a given amount of adrenalin, to draw any conclusions as to whether a given operative procedure has raised or lowered the tendency towards glycosuria.

Bearing these facts regarding the anatomy of the glands and the irregularity in adrenalin action in mind, let us see if there is any justification for the above conclusions regarding the opposing influences of thyroid and parathyroids.

Underhill and Hilsditch (37) in removing the thyroid, took great pains to avoid doing damage to more than one parathyroid on each side, and, as a result of observations on four

dogs, a rabbit, and a cat, they found that adrenalin produces glycosuria just as readily in thyroidectomized animals as in those that are normal. They concluded that the tendency towards glycosuria is unaffected by thyroidectomy, provided that two parathyroids at least are left intact.

Nor could the above described influence of thyroidectomy on the assimilation limit of dextrose be confirmed. Thus, in the same four dogs, Underhill and Hilsditch found that the subcutaneous administration of 5 grammes dextrose per kilo body weight did not cause glycosuria except in one of them, and in this one it was found that three parathyroids had been removed. These amounts of dextrose about represent the assimilation limit for dextrose administered in this way. Such results do not, however, contradict the view that the assimilation limit has been raised ; they only show that it has not been lowered. Marine and Lenhart have attacked the problem from a different standpoint. In Cleveland, as in most of the lake cities, nearly all dogs (95 per cent.) have enlarged thyroid glands in the various stages of hyperplasia. The assimilation limit in respect of dextrose, determined according to the method elsewhere described, was found not to differ from that of normal animals, at whatever stage of the pathological process the observations were made. In several of the dogs, in which the whole or a large portion of the thyroid gland was removed, no change was noted in the assimilation limit, provided not more than two of the parathyroid glands were injured. When this was the case, the tolerance for dextrose immediately fell. The administration of iodine to animals in which the thyroid is in a state of active hyperplasia very promptly causes the histological picture to change to one of colloidal involution ; but during this, no change was found to occur in the assimilation limit. Thyroid feeding, on the other hand, caused a fall, followed by a rise in the assimilation limit. Taking the above evidence as a whole, the verdict which we pronounce is that the parathyroid glands exercise a control over carbohydrate metabolism in such a way as to prevent the accumulation of excessive amounts of dextrose in the organism, but that the thyroid glands, in so far as carbohydrate metabolism is concerned, have no direct influence whatsoever.

The Relationship of the Pituitary Gland to Carbohydrate Metabolism.

For some days following removal of the posterior lobe (hypophysis) of the pituitary gland in dogs, polyuria and glycosuria are commonly observed symptoms (39). Gradually, however, the assimilation limit rises again, and ultimately it becomes distinctly higher than normal, so that the animals come to be able to tolerate large quantities of dextrose. On the other hand, when extracts of the posterior lobe are injected (intravenously or subcutaneously), either in normal dogs or in those from which the posterior lobe has been removed, a distinct lowering of the assimilation limit occurs, and this may be so pronounced as to cause glycosuria, even when the animal is on a normal diet (40). The interpretation which has been put on these observations, by Cushing and his co-workers, is that the posterior lobe (pars nervosa et intermedia) produces an internal secretion which depresses the utilization of dextrose in the organism. They explain the glycosuria that immediately follows removal of the posterior lobe as due to a hypersecretion which is stimulated by the manipulation of the gland during the operation.

Complete extirpation of the anterior lobe is not compatible with life,* but if a part only of this lobe be removed the dogs survive, and for some days after the operation they may be glycosuric (probably because of unavoidable manipulation of the posterior lobe during the operation). Later, however, the animals become normal in so far as carbohydrate metabolism is concerned. It is concluded that the internal secretion of the anterior lobe, although of such importance in controlling other types of metabolism, has nothing to do with that of the carbohydrates.†

In the earlier stages of acromegaly, a disease characterized by the abnormal growth of certain bones (lower jaw especially), there exists an irritative lesion which involves the anterior lobe of the pituitary gland, and to a certain extent also the posterior. The hypersecretion of the anterior lobe thus excited is believed to be responsible for the overgrowth of the bones and

* Horsley controverts this conclusion of Cushing. *Cf.* Swale Vincent: "Internal Secretions." Arnold (London), 1912.

† Injection of extract of anterior lobe also sometimes lowers the assimilation limit, but this effect is explained by Cushing, etc., as due to unavoidable incorporation of part of the pars intermedia in the extracts.

that of the posterior, for the tendency towards glycosuria which has been observed, especially by Borchardt, to exist in such cases (in 176 cases, 71 showed this symptom). After some time the lesion causes destruction, and when this has spread to the posterior lobe, a *raised* tolerance for sugar becomes evident. In the cases of acromegaly so far observed by Cushing, etc., this raised tolerance for sugar has been very pronounced, and has been interpreted as indicating that the cases are in a late stage.

When we consider the results of these observations as a whole, it is evident that certain of the ductless glands have an important relationship to the control of carbohydrate metabolism. The most important participators in this control are probably the adrenals, the pancreas, the parathyroids, and possibly also the posterior lobe of the pituitary gland. It is as yet very difficult to decide as to the exact rôle which each gland plays, but the simplest interpretation that can be put upon the results which follow the removal of one or other gland is that the organism has been thereby deprived of an internal secretion that is in some way necessary for the control of carbohydrate metabolism in the body. In the case of the parathyroids and the pancreas this hormone control must facilitate, and in that of the adrenals it must depress, the utilization of sugar. But when we attempt to particularize as to the *modus operandi* of these influences, we are met with insurmountable difficulties on account of the inadequacy of our knowledge concerning the history of sugar in the animal economy. We cannot, for example, say whether it is the glycogenic function or that of glycolysis upon which the " internal secretion " acts. Notwithstanding the many attempts to show such, no change in the supposed glycolytic power of drawn blood or of tissue extracts has been proved to be associated with removal of the glands ; it is, however, permissible to suppose that the glycogenic function is intimately associated in the intact organism with that of glycolysis ; that it precedes it, and therefore that the hormone control is centred on the glycogenic function. In favour of this view, the upsetting of the glycogenic function, which is so marked after pancreatectomy, and the profound effect produced on it by adrenalin, must be considered as strong evidence ; against it, the striking results obtained by Knowlton and Starling relative

to the influence of pancreatic extract on the glycolysis occurring in the blood of a perfused heart. The only way, however, by which light can be thrown on this extremely difficult problem is by the collection of accurate observations. Premature theorizing does more harm than good.

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CHAPTER V

THE GLYCOGENIC FUNCTION OF THE LIVER

A. Glycogenesis.

In all but one of the forms of experimental diabetes which have been at all accurately investigated, hyperglycæmia has been found to be dependent, in the first instance at least, upon an increased output of sugar from the liver, derived from the glycogen which is stored in the cells of this organ. The diabetes produced by phlorhizin is the exception. When the cause of the hyperglycæmia continues to act after all the available glycogen has been used up, the hyperglycæmia may, or may not, disappear. It depends on the nature of the cause; when this is nerve stimulation, such as in piqûre, or stimulation of the splanchnic nerves, the hyperglycæmia disappears; but when it is some change in the composition of the blood, such as that produced by removal of the pancreas, or by repeated injection of adrenalin, the hyperglycæmia continues. In such cases the sugar comes to be derived from protein, and the chemical process by which this glyconeogenesis occurs is one that has received much attention in recent years, and which is now pretty thoroughly understood. It will be discussed later (see p. 212), and meanwhile we shall proceed to study the glycogenic function of the liver—that is to say, the conditions under which glycogen becomes deposited in the liver, its source and manner of deposition, and the mechanism by which it becomes subsequently converted into sugar.

In order that we may investigate these questions, it is essential that we possess some strictly accurate method for the estimation of glycogen in the presence of the other constituents of the tissues in which it is contained. Much attention was given to this subject by Pflüger (1), who, after showing that the methods

employed by the older investigators were more or less unreliable, proceeded to evolve one which, besides being accurate, is comparatively simple, and—a most important point—takes but little time to carry through. Although we shall not describe this method in detail, it is important that we should consider the principles upon which it depends. The various steps in the process are as follows :

1. Liberation of the glycogen from the (liver) tissue.

2. Precipitation of the glycogen without any of the other constituents of the (liver) tissue.

3. Hydrolysis of the precipitated glycogen and estimation of the resulting dextrose.

Although glycogen is soluble in water, it is impossible, by hot-water extraction alone, to remove it all from the tissue in which it is contained. Two per cent. of alkali extracts more glycogen than water, but some loss occurs on account of its partial destruction (2), and because simultaneously extracted protein has to be separated from the extract (by Brücke's reagent) before the glycogen can be precipitated by alcohol. This cannot be done without the loss of some glycogen. With weak acid the extract contains less protein, but the glycogen becomes partially hydrolyzed.

When the glycogen-containing tissue is heated with 30 per cent. of caustic potash—*i.e.*, with an equal volume of a 60 per cent. solution—it has been found by Pflüger that the largest possible yield of glycogen is obtained, and that the protein substances undergo such a degree of destruction as renders them unprecipitable by alcohol. The duration of heating necessary to destroy the proteins varies according to the density of the tissue employed ; for liver, less than an hour suffices ; for muscle, about three hours should be employed. Prolonged heating does not, however, cause any destruction of the glycogen (3).

To complete the estimation, the digested mass is mixed in a beaker with an equal volume of water, and the glycogen is precipitated by adding an amount of ordinary alcohol (96 per cent.), corresponding to twice the volume of the diluted mixture. This is considerably more alcohol than is necessary to precipitate all of the glycogen. One half-volume suffices for this purpose, but by using such an excess the decomposition products of the protein remain in solution, thus rendering it unnecessary

to remove them by precipitation.* The glycogen is washed with alcohol and dissolved in water.

If the resulting solution of glycogen be colourless, as it should be,† its strength—*i.e.*, the percentage of glycogen—may be determined by means of the polarimeter. The specific rotatory power of pure glycogen is a D 196·57.

A more accurate method for its estimation consists in hydrolyzing the glycogen by heating with acid. For this purpose an amount of hydrochloric acid sufficient to give 2·2 per cent. in the mixture is added, and the solution heated in a flask connected with a reflux condenser for three hours (4) on the water-bath. The amount of dextrose in the hydrolyzed solution is then determined, either by the polariscope, or by any of the usual chemical methods used for this purpose. The amount of dextrose multiplied by 0·927 gives the amount of glycogen.

The alcohol precipitate is not pure glycogen. Besides a certain amount of ash, it contains glycogen-dextrines. These probably exist along with glycogen in the tissues, and, like the dextrines from starch, they differ from one another in their resistance towards caustic potash. The lower dextrines, like the sugars, are destroyed by this reagent, but the higher dextrines resist its action. These latter will accordingly become precipitated along with glycogen by alcohol. The readiness with which they are thus precipitated no doubt varies according to the molecular weight of the particular dextrine. With two volumes of alcohol, some of the dextrines which have escaped destruction by the alkali are not precipitated, but they become so when the filtrate, after removal of the glycogen, is mixed with ten volumes of

* After the precipitate of glycogen has settled, the supernatant fluid is carefully decanted off, and passed through a filter paper, so as to catch any particles of glycogen. The precipitate, which is still kept as much as possible in the beaker, is washed several times with 66 per cent. alcohol, and then with some absolute alcohol and ether, all the washings being passed through the same filter. If it requires further purification, the glycogen is dissolved in boiling water, and reprecipitated by alcohol.

When it is desired to obtain a glycogen preparation that is as free as possible of ash, it is well, at this stage, to subject a watery solution of the glycogen to dialysis against distilled water for a few days. We have adopted this as a routine when preparing glycogen for use in fermentation experiments.

To dissolve the glycogen it is usually advisable to leave as much of it as possible in the beaker, and then to pour boiling water on the filter, and allow this to collect in the beaker. When dissolved directly on the filter, much delay is likely to be caused on account of the slowness with which the colloidal solution filters.

† To obtain it colourless, it is advisable not to wait too long, after adding alcohol, before filtering.

alcohol. These facts indicate that the precipitate obtained with two volumes of alcohol is probably a mixture of glycogen and dextrines. It is therefore essential in comparative quantitative work to see to it that *exactly the same technique is followed for every estimation.*

We have found, when the above-mentioned precautions are taken, that the method is a fairly accurate one, as is shown in the following results of duplicate analyses :

Tissue Examined.	Per Cent. Dextrose of Glycogen.	Percentage Error.
Intact liver ..	4·149 4·149	0·0
	3·500 3·530	1·0
	10·310 10·087	2·0
	7·014 6·588	5·0
Liver extract ..	0·263 0·257	2·3

These figures are chosen from our protocols so as to illustrate the range of error when fresh tissue is employed. This is seldom more than 2 per cent. ; thus, in thirty duplicate analyses of intact liver, it was greater than 2 per cent. in nine cases, and was slightly over 3 per cent. in four. The cause of the error usually resides in the condition of the pieces of tissue taken for analysis, as regards the amount of blood and connective tissue which each contains. The error is much smaller when duplicate portions of minced tissue are examined. It is only with anatomically different portions of tissue that they occur ; thus, in duplicate samples of minced liver, Grube (5) found experimental errors of 0·76, 0·6, and 1·01 per cent. We have adopted 2 per cent. as the uncontrollable error. No doubt it could be considerably lessened by very painstaking manipulation, but we do not believe that the extra time and trouble would justify attempts at its curtailment. In the type of biochemical work discussed here, it is, we believe, far more important to submit many results that are almost correct rather than a few that are supposed to be strictly accurate. It must be remembered that there is, in any case, a large and variable biological error, which

is beyond our control, but which we can allow for by frequent repetition of the observations.

In the first place we must learn something regarding *the distribution of glycogen in the liver of the normal animal.* This knowledge is necessary in order that we may be able to study the effect which is produced by experimental or abnormal conditions on the glycogenic process. Such studies are usually made by removing a portion of liver from one lobe (after mass ligation) at the start of the experiment; this serves as the normal control. At various periods after the establishment of the experimental condition under investigation (*e.g.*, stimulation of a nerve, etc.) portions of other lobes are removed in the same manner as the first. The amount of glycogen in the various lobes is then determined, and, by comparison of the results, conclusions can be drawn regarding the rate at which the amount of glycogen is changing. When an increase in the amount of glycogen is occurring, we call it *glycogenesis*, and the opposite process, *glycogenolysis*. The difference in glycogen can be conveniently expressed as a percentage of the amount found in the normal control (percentile glycogenesis or glycogenolysis).

Quite apart from the experimental condition itself there are several influences, in every experiment of the type described above, which may cause changes in the amount and distribution of glycogen in the liver, and, to avoid errors, we must make ourselves familiar with these influences. A disregard of them is responsible for most of the confusion, as well as for the want of harmony, in a great proportion of the published work relating to the subject.

The fundamental data which we must obtain concern the distribution of glycogen over the liver (1) during absorption of food (*i.e.*, during glycogenesis), and (2) during the glycogenolysis, which is produced by anæsthesia and by death.

Although the liver is of exactly the same structure in its different lobes, it is possible that glycogen does not become deposited at an equal rate all over it, but that one portion may become filled up more quickly than another; in other words the mechanism which controls the deposition of glycogen (for example, the blood-supply) may not act equally on all parts. It has been stated that during the height of food absorption there is a greater amount of glycogen in the right lobes than in

the left, whereas when no absorption is in progress that the opposite distribution obtains (6), there being more in the left lobes. These differences in distribution are ascribed by Sérégé to imperfect mixing of blood in the portal vein ; the blood of the splenic vein, after entering the portal, so he states, remains more or less on the left side of the latter, and is distributed largely to the left lobes of the liver, whereas that of the mesenteric veins keeps to the right and supplies the right lobes. During absorption there will, of course, be glycogen-forming material in the blood of the mesenteric, but none in that of the splenic veins. Unfortunately Sérégé's results are open to severe criticism on account of the inaccuracy of the method (Fränkel method) which he employed for the estimation of glycogen. By the use of Pflüger's method, Grube (7) found that in twelve or more hours after taking excess of carbohydrate-rich food there was practically an equal distribution of glycogen over the liver. Although this shows that after absorption is over the distribution of glycogen is uniform, it does not throw any light on the nature of the distribution while absorption is in progress. This has been investigated by R. G. Pearce and the author. Five dogs, each weighing about 10 kilos, and as nearly as possible alike in age and breed, were starved for five days. On the sixth day each dog was fed by the stomach-tube with soup having cane-sugar dissolved in it, corresponding amounts of sugar being given to each animal. At varying periods after giving this food, the dogs were killed by chloroform, and the glycogen content of the various lobes of the liver determined.

The Table on p. 115 gives a summary of the results.

Quite contrary to expectation, the left lobes in the first five experiments contained the most glycogen. The fact, however, that this distribution was so regularly observed suggests that it must have been due to some cause, acting on the liver after death, and affecting the glycogen content of the different lobes to an unequal degree. The explanation of the difference is as follows :

After its removal, each piece of liver was placed in an Erlenmeyer flask, which was then weighed and allowed to stand until all the others had been similarly prepared. Although the caustic potash was then simultaneously added to all, post-mortem glycogenolysis must have occurred, during the twenty odd minutes which it took to complete the weighings, to a greater degree in

the *intact* liver than in the isolated portions (see p. 166.)* The experiment was therefore repeated in two dogs (Nos. 6 and 7 in the Table), with the difference that, immediately after its removal from the body, the liver was placed in a freezing mixture. In one dog (No. 6), six hours after feeding with cane-sugar, the greatest difference between the estimations amounted to 7·6 per cent., the largest amount of glycogen being found in the right lobe. In the other dog (No. 7), killed in twelve hours, the greatest difference was only 5·25 per cent., with the largest amount in the caudate and right central lobes. When every precaution against post-mortem glycogenolysis was taken, there was still a difference, amounting to from 5 to 7 per cent., in the

THE DISTRIBUTION OF GLYCOGEN IN THE LIVER.

Dog.	Time after Feeding at which Dog was Killed.	Per Cent. of Glycogen in Lobes.					Greatest Difference.	Greatest Difference in per Cent. of Largest Amount.
		Left.	Left Central.	Caudate.	Right Central.	Right.		
1.	1 hour ..	3·515	3·549	3·485	3·335	3·045	0·549	15·5
2.	3 hours ..	4·635	4·171	4·104	4·149	4·126	0·531	11·2
3.	5 hours (and 1 hour)†	3·481	3·468	3·312	3·094	3·054	0·427	12·2
4.	7 hours (and 3 hours)	9·393	10·026	10·198	9·721	8·970	1·228	12·04
5.	18 hours ..	7·525	6·708	6·725	6·801	6·700	0·825	10·9
6.	6 hours ..	10·845	10·890	10·710	11·350	11·610	0·900	7·6
7.	12 hours‡ ..	14·445	14·135	14·840	14·700	14·065	0·780	5·25

amount of glycogen in the different lobes; this difference was, however, no greater during absorption than at other times, nor did it behave in the manner described by Sérégé.

Having thus shown that during its deposition the distribution of glycogen over the liver is practically uniform, it remains for us to see whether it remains so during the opposite process—namely, when the glycogen is being broken down into sugar. The condition in which this glycogenolysis is most marked is that which supervenes on death. The distribution of glycogen at various periods after this has been found to behave as follows:

1. When the liver is removed from the body immediately after death, and the animal has not previously been under the

* This, we did not know of at the time of the experiment, and we relate our experience here for the benefit of other workers in the same field.

† The times given in brackets indicate when a second administration of food was given. ‡ Lobes removed from right to left.

anæsthetic for a prolonged period of time, the process of glycogenolysis is usually equal in intensity in different portions of the liver. This is shown in the following typical experiments :

(1) Rabbit killed by stunning, liver immediately excised, and placed in incubator.

Percentage Amount of Glycogen (Dextrose) in—

	Immediately after Death.	Twenty Minutes Later.
Quadrate lobes	11·05	10·31
Left lobes	10·80	10·21
Difference	0·25	0·10
Difference in per cent. of larger amount	2·26	0·97

(2) Dog died of hæmorrhage while under anæsthesia, and liver excised and placed in incubator.

Percentage Amount of Glycogen (Dextrose) in—

	Immediately after Death.	One Hour Later.	Two Hours Later.	Three Hours Later.	Four Hours Later.	Five Hours Later.	Six Hours Later.
Left lobe ..	4·675	4·016	3·726	3·610	3·756	3·450	3·233
Right central ..	4·620	4·165	3·866	3·570	3·230	3·186	3·033
Difference ..	0·055	0·151	0·140	0·040	0·526	0·264	0·200

(3) Dog died of hæmorrhage while under anæsthesia, and liver excised and placed in incubator.

Percentage Amount of Glycogen (Dextrose) in—

	Immediately after Death.	Fifteen Minutes Later.	Thirty Minutes Later.	Forty-five Minutes Later.
Left lobe	3·78	2·98	2·62	2·38
Right central	3·82	2·89	2·61	2·40
Difference	0·04	0·09	0·01	0·02

Occasionally, however, glycogenolysis does not proceed uniformly over the liver, even when this is placed in the incubator. Thus, in a rabbit's liver, treated exactly like that described above, the following percentage amounts of glycogen were found :

	Immediately after Death.	Twenty Minutes Later.	Fifty Minutes Later.
Quadrate lobe	8·840	8·111	6·733
Left lobe	8·793	7·960	5·932
Difference	0·047	0·151	0·801
Difference in per cent. of larger amount	0·530	1·860	11·800

2. When the liver is left in the body after death, glycogenolysis proceeds more quickly in the deeply lying lobes than in those that are in front. The cause of the difference is mainly inequality of temperature. In some cases it may also be partly due to differences in blood-content, on account of hypostatic congestion of the posterior lobes. Thus, in a dog killed by bleeding and then left on a warm table, the temperature, in about half an hour after death, between the front lobes was 30° C.; between the deep lobes it was 36° C., and the glycogen content was as follows:

	Immediately after Death.	Ten Minutes Later.	Twenty Minutes Later.
Front lobes	7·175	7·664	7·370
Back lobes	6·750	7·025	6·234
Difference	0·445	0·639	1·136
Difference in per cent. of larger amount	6·200	8·300	15·400

The glycogenolysis which is produced by ether anæsthesia is not usually so rapid as that which follows death (see p. 189), but, like this, it does not always proceed at a uniform rate through the liver.

Thus, in a dog that had been under ether for three hours, and in which there had been no operative interference with the abdomen, the following percentage amounts of glycogen (dextrose) were found in the various lobes: Left, 2·506; left central, 1·650; right central, 1·635; right, 1·551. The greatest difference was 0·955 or, in per cent. of the largest amount, 38·2.

In another observation of the same kind the following results were obtained: Left lobe (back), 3·290; left lobe (front), 3·140; left central, 3·120; caudate (back), 2·760; caudate (front), 2·990; right central, 3·180; right, 3·070; spigelian, 2·800. The

greatest difference was 0·530, or, in per cent. of the largest amount, 16·1.

The deeper lying lobes (when the animal is on its back) contain the lesser amounts of glycogen.

These facts must, of course, be kept in mind when it is desired to make comparisons in etherized animals of the rate of glycogenolysis in the liver under different experimental conditions.

Knowing, then, what degree of physiological variation we are to expect in the distribution of glycogen in the liver, we may now proceed to consider *the sources of glycogen*—that is to say, the nature of the foodstuffs which, by their absorption into the blood of the portal circulation, cause an increase in the amount of glycogen in the liver. There has been a great amount of interest shown in this subject, because of the practical application of the knowledge in the treatment of diabetes mellitus. The essential thing to be aimed at in this treatment is a diminution in the amount of sugar in the blood, not merely in order that there may be a corresponding diminution in the glycosuria, but also in order that the deleterious influence of an excess of sugar in the organism may be diminished and the pathological changes which result from it warded off. Moreover, an improvement in the assimilative powers for carbohydrates usually follows the reduction in the amount of circulating sugar. If a certain foodstuff forms glycogen during health, it will of course form sugar during diabetes, and in this disease such foodstuff must be prohibited. It is probably correct to look upon the milder cases of diabetes (the so-called "glycosuria e saccharo" and "glycosuria ex amylo") as being due to a sluggishness in the glycogenic function of the liver—as being due, namely, to a failure on the part of this organ to remove from the portal blood that portion of absorbed carbohydrate which is not immediately required in the blood of the systemic circulation. In the severer cases of the disease the sluggishness gives place to a more complete failure of this function, and other perversions of metabolism become developed, which make it increasingly difficult for the tissues to assimilate the sugar.

To ascertain which foodstuffs tend to glycogen formation, we may adopt one of two general methods. The one of these, called the *direct*, consists in first of all ridding the liver of glycogen, then feeding with the foodstuff under investigation, and, after

some time, killing the animal and determining the amount of glycogen in the liver (and muscles). The other, called the *indirect*, consists in observing the amount of sugar in the urine, following the giving of a certain food, in an animal that has been rendered completely incapable of utilizing it—that is to say, in a diabetic animal. If the foodstuff forms sugar under these conditions, it is assumed to be capable of forming glycogen in health (see, however, p. 218).

Since the direct method is now more or less obsolete, and on account of the fact that there already exist several exhaustive reviews of the results (1 and 9) that have been obtained by use of it, we shall do no more here than very briefly review the main conclusions it has led to. The indirect method will be discussed later.

The first step in the direct method consists in ridding the liver of glycogen, and the success of the investigation depends largely on the thoroughness with which this is done. Several methods may be employed for this purpose. Starvation alone cannot be depended on, because, after an initial reduction to a minimum, there may subsequently occur a reaccumulation of glycogen, on account of the extensive dissolution of the body protein, for protein, as we shall see later, forms glycogen. Muscular exercise, especially if combined with starvation, is a much more certain means of using up the glycogen. Besides voluntary exercise, muscular contractions can be experimentally produced by means of small doses of strychnine, after taking precautions to prevent asphyxia ; or we may keep the starved animals in a cold place so as to excite increased muscular tone (see p. 193).

All carbohydrates which lead to an increase in the percentage of dextrose or lævulose in the portal blood cause glycogen to be deposited in the liver. Galactose may possibly also form it, but this has not been so definitely shown by the direct method as for the other monosaccharides. It must be remembered that this sugar differs in some of its fundamental characteristics from dextrose and lævulose ; it does not, for example, ferment with certain forms of yeast. Still, since it is utilized in the body when administered subcutaneously, and since it is converted into dextrose in the diabetic animal, it is no doubt a glycogen-former. In the intestine of all animals there are suitable ferments for the conversion of maltose and cane-sugar into dextrose and lævulose, so that these disaccharides are striking glycogen-

formers. On the other hand, in many animals (*e.g.*, the herbivora) lactose does not become hydrolyzed because of the absence of the enzyme lactase in the intestinal juice, so that no glycogen formation occurs when this sugar is taken. When lactase is present, as in suckling animals and in most of the omnivora, the lactose is split into dextrose and galactose, and there is glycogen is formed.

When the dissaccharides as such gain entry to the blood, as by parenteral injection, or when they are absorbed as such from the intestine, they form glycogen according to whether or not they become hydrolyzed in the blood. None forms glycogen directly. Of the disaccharide-splitting enzymes, the only one present in the blood, under ordinary conditions, is maltase, consequently maltose is the only disaccharide which causes glycogen formation on parenteral injection. Cane-sugar and lactose, on the other hand, are immediately excreted in the urine (see p. 202). Enzymes capable of splitting cane-sugar and lactose may, however, be caused to appear in the blood by injecting solutions of these sugars subcutaneously or intravenously (10). Under such conditions, the above sugars can be retained by the animal when they are injected in the blood.

So far the results of the direct method are definite and dependable, but when we attempt to employ it for the purpose of ascertaining whether such substances as the pentoses or the proteins can cause glycogen to be formed, we do not, as a rule, succeed in obtaining satisfactory results. Probably the only experiment by the direct method in which it has been shown that protein can form glycogen is one performed by Pflüger. This investigator succeeded in showing in fasting frogs that the glycogen content of the entire body rose from week to week. Errors due to differences in individual frogs were eliminated by using large numbers of animals.

All that we learn from the results of the direct method is whether or not the ingestion of a certain foodstuff causes glycogen to be formed in the liver ; but this is all. We learn nothing as to how this synthesis is accomplished—that is to say, whether it is solely a function of the liver, or whether there may not be necessary, for its accomplishment, some preliminary change in the monosaccharide molecule, as might be effected by an action on it, either during its absorption through the intestinal epithe-

lium, or after it has gained entry to the blood, by some agencies present in this fluid; for example, by some hormone or enzyme.

Crofton (11) described such a preliminary change as occurring during absorption from the intestine. He came to this conclusion because he thought that glycogen was deposited in the liver when dextrose was absorbed from the intestine, but not when dextrose was injected directly into the blood of the portal circulation. Disregarding for the present the results of other experimenters, which directly contradict those of Crofton (*cf.* Pflüger, 12), we may state here that even were the conclusions of this author true, this could certainly not be deduced from the experimental protocols which he offers as evidence. There are, for example, only four experiments recorded in which the dextrose was injected directly into the portal circulation through a cannula inserted in a mesenteric vein. Two of these experiments on dogs that had not fasted must be discounted, because the liver contained, to start with, a high percentage of glycogen (in one case 10 per cent.; in the other 13·9 per cent.), and could quite possibly not have deposited more under any circumstances. Only two injection experiments were performed on fasting dogs, and in both of these a slight decrease of glycogen—viz., 2·3 per cent. in the one case, and 1·4 per cent. in the other—occurred. These results do not indicate that no glycogen could be formed from injected sugar, for during the period elapsing between the removal of the first and second portions of liver, the animals were subjected to an operation—the introduction of a cannula in a mesenteric vein (under local anæsthesia ?). There must have been emotional excitement, and the glycogenolysis, which is stimulated by this (see p. 54), coupled with the natural glycogenolysis which must have occurred in the time elapsing since the removal of the first portion of liver, must have caused a considerable decrease in the glycogen content of the liver. If any conclusion at all can be drawn from his meager results, it must certainly be exactly the opposite to that drawn by Crofton. The injected dextrose must have formed about as much glycogen as was meanwhile being discharged as dextrose into the systemic blood.

To investigate the other possibility—namely, that the liver in building up glycogen must be aided by the co-operation of

other agencies—for example, by the presence in the blood of some hormone derived from a ductless gland—the most convincing type of experiment consists in determining the behaviour of its glycogen content during artificial perfusion of the liver outside the body. If it should be found that glycogen is formed during perfusion of the liver with blood that is not simultaneously circulating through other parts of the body, it would be strong evidence that this viscus is capable, in itself, of forming glycogen. It would not finally prove this, however, for there might remain in the blood, even after it has been for some time removed from the body, a sufficient amount of hormone to account for the synthesis. The undoubted relationship between certain ductless glands and the glycogenic function of the liver are reasons why it is important to see whether, in their absence, the liver is capable of producing glycogen.

Unfortunately, however, successful perfusion of the liver is fraught with many difficulties. This is particularly the case in warm-blooded animals ; in cold-blooded animals, on the other hand—for example, in the turtle—it can be much more successfully performed.

The first of these difficulties is that we have no means of knowing in what state of physiological activity the liver may be during the perfusion. Of its many functions, only some may be resistant enough to remain active during perfusion ; others may cease immediately the normal circulation is interfered with. There is no visible indication of the functional integrity of the liver, as there is of the heart, for in this case we can observe the beats. But what are we to take as the criterion of successful perfusion in the case of the liver ? The secretion of bile is inadequate, for even though it should be shown—which it has not—that the bile thus secreted is normal in composition, this may be the least vulnerable of the many functions of the liver. Certain other functions, such as the synthesis of ethereal sulphates and the formation of urea, are certainly retained during perfusion, but these are comparatively simple chemical processes, and they do not involve, as does the formation of glycogen, the deposition of masses of colloidal substance within the protoplasm of the cells. Probably the most sensitive test of the successful artificial perfusion of the liver is therefore the behaviour of glycogen. If the perfusion is successful, there should be no more than a slight diminution in the

glycogen content, and sugar should not accumulate in the perfused fluid.

Grube (13) perfused the liver of the dog and cat by the use of Brodie's apparatus. He left the viscus *in situ* during the perfusion so as to avoid kinking of the vessels, and he started the perfusion through a branch of the portal vein before killing the animal so as to avoid any cessation in the blood-flow. He did not, however, succeed in preventing glycogenolysis, for sugar accumulated in considerable quantity in the perfused fluid, and although he thought that in some of the livers a certain amount of glycogen had been formed, examination of his results does not bear this out, when allowance is made for the very large experimental error involved in the method which he employed for estimating the glycogen.

Adopting all the precautions suggested by Grube, and introducing what we believed to be improvements of our own, R. G. Pearce and the author (14) have tried repeatedly so to perfuse the dog liver as to prevent post-mortem glycogenolysis, but have utterly failed. We are convinced that it is impossible, by the ordinary methods of artificial perfusion, to prevent glycogen breakdown in the liver. We have come to this conclusion only after repeated attempts, of which the following is a brief account. The apparatus which we employed—constructed by my technician, Mr. E. Warnick—permitted us to fulfil the following conditions :

1. The blood-mixture (usually equal parts defibrinated dog blood and Locke's solution), containing about 0·2 to 0·5 per cent. dextrose, was perfused through the portal vein at a perfectly constant pressure, which was regulated by means of a water-valve. The pressure chosen to start with was always equal to 10 millimetres Hg. Frequently, however, with so low a pressure, the rate of perfusion was very slow, even at the start, and in all cases it become progressively slower during the perfusion, presumably because of œdema of the hepatic cells. To overcome the increased resistance the portal pressure had frequently to be raised, but the liver invariably became abnormal in appearance when the pressure rose above 20 millimetres Hg. The endeavour was made to perfuse at the rate of from 100 to 180 c.c. per minute. In order to facilitate the perfusion and at the same time simulate as closely as possible the normal conditions, a suction pressure, which was interrupted about three

or four times a minute, was applied to the vena cava. This pressure usually amounted to about 20 millimetres Hg. In some of the experiments also, simultaneously with the low-pressure perfusion in the portal vein, perfusion through the hepatic artery at a pressure of 100 millimetres Hg was performed. The hepatic artery blood was highly oxygenated, that through the portal vein moderately so. The remarkably early development of the resistance to perfusion in the portal circuit, which developed* in the majority of the experiments, gave the impression that the blood and Locke's solution mixture might be exerting a toxic action on the hepatic cells, and that this was the cause of their swelling. To counteract such a chemical action of the blood-mixture various expedients were tried : thus, adding small quantities of alkali (Na_2HPO_4) to the perfusion fluid so as to neutralize any acids produced by autolysis, employing undiluted defibrinated blood, making the blood very arterial, etc., but all without avail—that is to say, there was no improvement in the rate of perfusion as a result of these modifications. The only method by which we could be certain of having an adequate perfusion at low pressure was by using Locke's solution with only a small proportion of blood in it, but then the glycogenolysis was extreme. In some experiments, for no evident reason, the perfusion would proceed fairly satisfactorily, but a repetition of the perfusion, under exactly the same conditions, would yield utterly unsatisfactory results.†

After trying in all over twenty experiments, we decided to abandon further attempts, especially since, as the following brief survey of our observations on the behaviour of glycogen will show, we could not definitely prevent glycogenolysis. Because we have failed, we cannot, of course, assert that it is impossible successfully to perfuse the mammalian liver outside the body, but we do not believe that it has ever been satisfactorily performed, and we are confident that it will require some radical modification in the technique usually employed in such experiments before it is so. In the following Table all experiments are omitted in which the liver became markedly œdematous as a result of too high a portal pressure.

* In some of the experiments we believe that this was due to intravascular clotting in the liver. Before its entry in the portal vein, the blood-mixture was always filtered through gauze.

† De Meyer's statement that Locke's solution containing some arterial blood can be perfused without any glycogenolysis occurring is certainly incorrect.

TABLE OF THE MORE SATISFACTORY PERFUSION EXPERIMENTS.

No. of Experiment.	Type of Perfusion.	General Result of Perfusion.	Per Cent. Glycogen at Different Times.	Change in Blood-Sugar (per Cent.).	Change in Ferment in Blood.	Remarks.
8.	Blood + Locke's solution in portal vein	Flow satisfactory ..	10 per cent. disappeared in 90 minutes	0·456 at start; 0·415 at end	Did not change	The blood used had been perfused once or twice through another liver before starting.
9.	Ditto	Flow became very slow	6·85 at start; 3·90 in 120 minutes	0·165 at start; 0·161 at end	—	The difficulty in perfusion in this case probably due to clotting.
11.	Blood alone	90 c.c. per minute soon after start; 65 to 70 c.c. towards end	9·79 at start; 8·85 in 56 minutes; 7·115 in 120 minutes. ∴ 19·6 per cent. disappeared	0·269 at start; 0·298 at end	Very slight increase	—
14.	One part Locke's solution, two parts blood	Flow fair	1·74 at start; 1·15 at end	—	No change	—
15.	Blood + Locke's solution in portal vein and hepatic artery	100 c.c. per minute soon after start; 180 c.c. per minute during most of experiment	7·37 at start; 6·925 after 175 minutes	0·120 at start; 0·187 at end	—	A piece of liver kept in abdomen near liver during perfusion showed no disappearance of glycogen.
17.	Ditto	Average flow of 128 c.c. per minute	4·03 at start; 2·0 in 75 minutes; 0·65 in 140 minutes	0·111 at start; 0·135 at end	Distinct increase	Towards end of experiment the blood was allowed to become venous; the rate of perfusion *increased*.
18.	Ditto	Flow very unsatisfactory	Practically no glycogen present	0·147 at start; 0·172 at end	—	The hepatic artery perfusion did not proceed satisfactorily; 500 c.c. blood, however, was perfused through this path.
20.	Blood alone in portal vein and hepatic artery	Ditto	3·45 at start; 0·85 in 110 minutes	—	—	In a portion of liver that was ligated, the glycogen at end was 2·5 per cent.
26.	Blood + Locke's solution + Na_2HPO_4 solution	100 c.c. per minute throughout	3·40 at start; 2·45 after 60 minutes; 1·90 after 100 minutes	0·129 after 70 minutes; 0·129 after 100 minutes*	—	* Some Locke's solution was added to blood to make up for losses.

In one experiment only (No. 15) was there no material disappearance of glycogen, but we can scarcely credit this result to successful perfusion, for a portion of the same liver, removed and kept in the abdomen during the perfusion, also retained all of its glycogen. Nor were we able to obtain the same result again by adopting the same technique (viz., independent perfusion of the portal vein and hepatic artery). In some of the other experiments, only slight glycogenolysis occurred (Nos. 8, 11), but since we could never predict, from the behaviour of the perfusion, of what degree the glycogenolysis would be, it was obvious that the whole method was utterly useless for our purpose.

J. de Meyer (15) attempted to show glycogen formation in the dog's liver by an entirely different method. Recognizing that it is impossible to prevent glycogenolysis by perfusing Locke's solution, and that it is therefore inaccurate to compare the glycogen content before and after perfusion, this author proceeded to perfuse the right and left lobes of the liver separately, the one with Locke's solution alone, the other with Locke's solution containing an excess of dextrose. It was argued that a comparison of the glycogen content in the two halves of the liver would reveal whether glycogen formation had taken place, for although glycogenolysis would of course occur in both halves, it would be lessened in the one of these by a simultaneous glycogen formation. Three experiments of this nature are recorded with the following results :

Glycogen Content in per Cent.

A.—In Portion perfused with Locke's Solution.	B.—In Portion perfused with Locke's Solution and Dextrose.	Duration of Perfusion.
(1) 0·433	1·195	1¼ hours
(2) 8·08	8·56	1½ ,,
(3) 0·0304	0·0456	—

From what has already been said regarding the estimation of glycogen in different portions of the liver, it is evident that two of these results (Nos. 2 and 3) cannot be considered as showing glycogen formation. The remaining result (No. 1) is also not at all convincing, for the percentage of glycogen recorded is low, and we are not assured, by duplicate analyses, that the estima-

tions are correct. Moreover, no such observations as these are of any value until it has been shown that when both halves of the liver are perfused with the *same* fluid they contain the *same* amount of glycogen. This is assumed by de Meyer ; it is certain that it is very seldom the case (p. 117). Quite apart from the evident fallacies in de Meyer's work, it is open to question whether the principle of the method of double circulation is correct, for, in the presence of such excessive post-mortem glycogenolysis, it is difficult to see how much glycogen formation could occur.*

Although it is apparently impossible to show that the isolated mammalian liver can form glycogen, it is said that definite evidence of glycogen formation can be obtained, under certain conditions, in the isolated liver of the turtle (Grube, 16). In this animal the liver consists of a right and a left lobe, connected together by a narrow bridge of hepatic tissue, each lobe being supplied by a branch of the umbilical vein. By removing one lobe at the start of an experiment, perfusing the remaining lobe with Ringer's solution (containing dextrose, etc.) for some time, and then comparing the glycogen content of the two lobes, conclusions can be drawn regarding the behaviour of the glycogen during the perfusion. The method is, however, not without serious imperfections, because of a fairly rapid glycogenolysis which occurs, and which of course materially masks any new formation of glycogen in the perfused lobe. Moreover, this glycogenolysis does not occur at an equal rate in the liver of different turtles. In order to eliminate these sources of error, Grube introduced another method, in which a cannula was inserted in each branch of the umbilical vein, through which the one lobe was perfused with Ringer's solution alone, and the other with Ringer's solution containing the substance whose influence on the glycogen content of the liver it was desired to investigate. With all the conditions of perfusion identical in both circuits, it was hoped that any breakdown of glycogen on the one side would occur to the same degree on the other ; and that, under such circumstances, any observed difference could be ascribed to glycogen formation out of the added substance. Both methods depend on the assumption that the two lobes of the turtle liver contain the same percentage of glycogen at the

* De Meyer also describes experiments in which the entire liver was perfused with Locke's solution. The results are, however, of no value, and can be disregarded.

start of the perfusion. But this is not the case, for, in the livers of seven turtles examined by Schöndorff and Grebe (17), the differences between the two lobes amounted to from 8 to 32 per cent., and in no case were equal amounts of glycogen found present.* In several turtle livers which we have examined in the same way, similar differences between the two lobes were invariably found to exist. Grube himself admits, in a recent publication, that there is not entire agreement between the two lobes. In the case of four livers examined by this author, the percentile differences were: 2·1, 9·5, 6·7, and 3·3. It is usually the left lobes that contain the larger amounts.

Besides this source of error, another exists in the case of the method in which the two lobes are separately perfused, on account of there being a more rapid glycogenolysis in the one lobe than in the other. The difference in glycogen content of the two lobes therefore becomes exaggerated as a result of perfusion; thus differences amounting to 36·8, 22·7, and 33·1 per cent. have been obtained by Grube, values that are *many times* greater than those obtained before perfusion. Notwithstanding these severe criticisms, Grube asserts that the method yields evidence of glycogen formation, not only for the monosaccharides and glycerine, but also for formaldehyde, the lowest member of the aldehyde group. For amino-acids, however, no evidence of glycogen formation could be obtained. Typical results for the sugars are as follows:

Variety and Amount of Sugar employed.	Glycogen per Cent. in Liver.		Percentile Increase of Glycogen.
	Before Perfusion.	After Perfusion.	
Dextrose, 6 grs. ..	0·95	3·80	30·0
Lævulose, 24·7 grs. ..	3·49	6·45	84·9
Galactose, 12 grs. ..	3·60	4·30	19·4
Lactose, 24 grs. ..	5·59	5·04	—
Saccharose, 18 grs. ..	0·16	0·17	—

It is evident that glycogen was formed only when dextrose or lævulose was added to the perfusion fluid.

Concordant results have been obtained by Nishi (16A). But when formaldehyde was added, very doubtful results were obtained, results which are certainly within the experimental error of the method. In the experiments (19) in which the two

* The actual percentile differences were: 10·8, 13·0, 14·3, 15·4, 32·3 more in left lobe, and 8·0, 8·8 in right.

lobes were perfused, a surplus of glycogen exceeding 30 per cent. was observed on the formalin side in five out of twelve cases (19 and 20). By the other method there was, in two out of three cases, actually less glycogen in the half of the liver after perfusion with formalin than in the half which was not perfused (20).

A careful repetition of Grube's work by Schöndorff and Grebe (17) led them to the conclusion that there is no evidence that formaldehyde can form glycogen in the turtle liver. In only two out of fifteen experiments was there more glycogen in the half of the liver that had been perfused with formaldehyde, and the differences in these two cases were within the experimental error involved in the estimation. Both types of experiments described above were employed.

On account of the very large variations which are thus shown to exist in the glycogen content of the two lobes of the turtle liver, and on account of the fact that there are some difficulties in the estimation of glycogen in this liver (21), at least, when the polariscopic method is employed, it will be necessary to repeat even the experiments with monosaccharides.

If it could be demonstrated that the liver of any animal is able to build up glycogen out of formaldehyde, it would constitute one of the most important discoveries of biochemistry, for it would betray a synthetic power in the animal kingdom that is not far short of that of plants. It is believed that in plants formaldehyde is the first product formed in the green leaf by interaction of carbon dioxide and water under the influence of energy derived from the sun's rays. Several molecules of formaldehyde then fuse together, by a process of condensation, to form sugars. Ferments control this condensation so that only optically active sugars result. If it could be shown that the animal cell can perform this condensation, it would mean that it is possessed of much greater synthetic power than it is commonly credited with. But apart from this, the discovery would also be of tremendous import in connection with the intermediary metabolism of proteins and fats, for it would explain very simply how carbohydrates might be derived in the organism out of either of the others. Both proteins and fats could undoubtedly contribute to the formation of formaldehyde which would then condense in the liver to dextrose. But it is useless to speculate farther. For the present the important question is, Can formaldehyde become condensed to dextrose (glycogen) in the animal

cell ? So far, the answer must be that there is no evidence that it can.

Whether or not the liver can produce dextrose by synthesis from formaldehyde, it certainly can combine dextrose molecules into glycogen in the intact animal, and apparently (if Grube's observations on the turtle liver be reliable) it can perform this synthesis independently of the rest of the organism—that is to say, it is not necessary that the dextrose molecule be prepared by some other metabolic agency before the liver can condense it to glycogen.

This brings us to inquire as to the nature of the agency which produces the glycogen in the liver. We shall see later that the glycogen stored in the liver becomes broken down to dextrose, as the result of the action of an intracellular enzyme called " glycogenase." The fact that certain other enzymes, such as lipase, can, under certain conditions, reverse their usual action of accelerating hydrolytic decomposition to one of synthesizing, has opened the question whether the formation of glycogen may not be because of a reversible action of glycogenase.

The chances for the existence of such an action on the part of glycogenase do not appear nearly so bright as they did when they were first mooted some years ago ; for at that time it was thought to be a firmly established fact that the disaccharide ferment maltase can, under certain conditions, reverse its better-known action of decomposing maltose to dextrose to one in which it recombines the dextrose molecules back again to maltose. But it has been shown that it is not maltose, but its isomeride, isomaltose, which results from this synthesis, and upon this disaccharide maltose has no hydrolytic action.

However, some attempts have been made to see whether glycogenase can synthesize glycogen.

Cremer (22) allowed press juice of yeast to stand for about eight hours at room temperature, by which it became glycogen-free ; at least, it did not give the iodine test for glycogen. He then added 10 per cent. or more of fermentable monosaccharide to the fluid, and after twenty-four hours found that an iodine test for glycogen had been acquired by the mixture. His best results were obtained by adding 30 per cent. lævulose (Schering's), and allowing the mixture to stand for sixty hours at 10° to 12° C.

I have searched in vain in the literature for any further publi-

cation, from this or any other author, that would confirm this result. It certainly cannot be accepted as conclusive, partly because it is not in any way quantitative, and partly because the iodine reaction for glycogen is by no means sufficiently reliable for such work.

Reactions which are capable of being influenced by enzymes are such as would also proceed, although ever so slowly, in their absence. They are balanced reactions—*i.e.*, the action can proceed either in the direction of building up or breaking down until a certain equilibrium or balance becomes established between the two sides of the equation. The enzyme can merely affect the rate of the change towards the equilibrium-point. The chemical reaction involved in the building up and breaking down of glycogen is hydrolytic—*i.e.*, it consists in the absorption or liberation of a molecule of water. For such a reaction the equation is as follows:

$$\text{Glucose} + \text{glucose} \rightleftarrows \text{glycogen} + \text{water}.$$

Since this is a balanced reaction, it must obey the law of mass action which is expressed in the equation—

$$\frac{\text{C. (glucose)} \times \text{C (glucose)}}{\text{C. (glycogen)} \times \text{C (water)}} = \text{constant},$$

where C indicates that gramme molecular quantities are used.

From this second equation, it follows that if any one substance in the reaction changes in amount, all of the others must also become changed so as to keep the balance. All of the reactions on which ferments have an influence take place in the presence of water, so that there is a constant excess of this substance present; consequently the equilibrium-point must stand very near the right-hand side of the chemical equation—that is to say, there can be but very little glycogen present when the equilibrium-point is reached. Unless there is some provision for removing the glycogen, it is evident, therefore, that very little of it could be formed by the action of glycogenase in a solution of glucose. But of course conditions may be such in the liver that removal of glycogen from the reaction occurs, by its becoming combined as it is formed within the protoplasm of the liver cell.

The transformation of glucose to glycogen is not, however, so

simple as the above equation would indicate, for there are several intermediary substances, namely, maltose and the dextrines. We do not know how many of these there may be, but there are certainly several (see p. 111). The first stage in the synthetic action should therefore be written :

$$\text{Dextrose} + \text{dextrose} \rightleftarrows \text{maltose} + \text{water};$$

and the second stage,

$$\text{Maltose} + \text{maltose} \rightleftarrows \text{dextrine I} + \text{water};$$

and the dextrines would then have to combine together in some way to form glycogen. However this combination of dextrines may be brought about, it is highly improbable that enough of maltose could ever be formed to allow of even the simplest dextrine being produced ; much less, therefore, could we expect the formation of any glycogen in such an experiment. If any evidence of the synthetic action of glycogen is to be expected, we must at least start with a mixture of suitable dextrines and maltose.

Another condition which must be kept in mind in such an experiment is that the proper isomerides of the various sugars (and polysaccharides) are present in the mixture. We have already seen that there are two distinct varieties of dextrose (α and β) ; so are there of maltose (maltose and isomaltose), and we have called attention to the probable significance of these facts in the metabolism of the sugars (see p. 84).

Taking all these considerations into account, we have attempted to demonstrate the synthesis of glycogen by glycogenase in the following experiment :

Five grammes dried glycogen, prepared as pure as possible from dog liver, was dissolved in 110 c.c. water, and mixed with 10 c.c. of a saline extract of blood-free liver. The flask containing this mixture was hermetically sealed, and kept at about room temperature. (There was a considerable sediment in the flask on the second day and subsequently.) Five c.c. of the contents of the flask were removed on each of the two succeeding days and precipitated with alcohol, the reducing power of the precipitate (after hydrolysis) and of the filtrate being then determined. It was found that most of the glycogen had disappeared on the second day. At this stage, therefore, a mixture of glycogen precursors must have been present in the flask. To this mixture was then added 10 grammes of maltose, the flask being

again sealed after removing 5 c.c. of its contents for determination of alcohol-soluble and alcohol-insoluble substance. Similar samples of 5 c.c. were removed every day or so for this purpose. Great care was taken always to use exactly the same amounts of alcohol, both for precipitating and for washing the precipitates.

The following amounts of reducing substance (in 5 c.c.) were found :

Day.	Sugar in Alcohol Precipitate.	Sugar in Solution (Filtrate and Washings).
1	0·1460	Trace
3	0·0568	—
4	0·0450	0·032
	0·0830*	0·176
5	0·0560	—
10	0·0570	—
11	0·0660†	0·148
	0·0558[1]	0·278
24	0·0350	0·212
32	0·0342	0·205
46	0·0333	0·260

* Ten grammes maltose added. The maltose in 10 per cent. solution gave a precipitate with alcohol.

† Reaction acid, so was neutralized and more liver extract added. This changed values to that marked with [1].

Although by no means a perfectly controlled experiment, yet the results obtained on the glycogen precipitate show quite conclusively that no new formation of this substance had occurred.

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CHAPTER VI

THE GLYCOGENIC FUNCTION OF THE LIVER (*Continued*)

B. Glycogenolysis

We have seen that the glycogen stored in the liver becomes gradually converted into dextrose, which is discharged into the blood of the vena cava, according to the needs of the organism for carbohydrate. The possible nature of the mechanism which is responsible for this adjustment has been discussed, but we have as yet learned nothing regarding the nature of the agency in the liver by which the conversion of glycogen into dextrose is effected, or of the means by which this agency can be made to undergo variations in activity in response to nervous or hormone control. Such knowledge is, however, necessary in order that we may understand what it is that goes wrong in those conditions in which the liver discharges excessive amounts of dextrose, and so causes hyperglycæmia. It has already been pointed out that in certain forms of experimental diabetes there ceases to be any hyperglycæmia after the liver has been depleted of glycogen; whereas in others the hyperglycæmia does not disappear, but the liver continues to produce dextrose because of the formation of this sugar out of substances which are not themselves carbohydrate in nature. This process of "glyconeogenesis," as it is called, supervenes on one of excessive glycogenolysis; the one disturbance merges into the other, so that it is probable that the same defect in metabolism is responsible for both. In other words, the underlying lesion of diabetes affects the glycogenolytic process alone in the earlier stages of the disease, but in the later stages it involves the glyconeogenic as well.

A knowledge of the cause of glycogenolysis is of importance, therefore, not only on account of the transient hyperglycæmias, but because it may guide us to an understanding of the cause of glyconeogenesis; and, knowing this, we may be enabled to

place our finger on the fault in the metabolic process that is responsible for diabetes. It is not to be understood that every form of diabetes starts in the same way ; indeed, many of those who have clinical experience with the disease believe that the initial disturbance may be different in different cases. On the other hand, be the initial cause what it may, it exerts its influence first of all on the glycogenolytic function of the liver.

For many years it was a much-debated question whether the process of glycogenolysis depends on the vital activity of the liver cell or on the action of an enzyme secreted by it. These were the days when a sharp distinction was drawn between organized and unorganized ferments—between those fermentations, namely, that require for their consummation the absorption of the substance to be acted on into the protoplasm of the living cell, and those that can proceed in the absence of living cells, although influenced by an enzyme secreted by them. We now know that the agency which affects the change is the same in both classes of fermentation—that it is an enzyme which in the one case remains within the body of the cell that produces it, but is secreted outside it in the other. In the former case, before the enzyme can unfold its action, it is necessary that the substance to be acted on should become absorbed into the body of the cell. This absorption is possible only so long as the cell is alive, and it will vary according to the ability of the cell to absorb the substance. The activity of the intracellular enzymes must therefore be much more susceptible to the influence of outside conditions than is that of the extracellular enzymes ; for it will be subject not only to the conditions which act on the enzymic process itself, but also to those which effect the absorbing power of the cell.

The outcome of the older observations was the discovery that certain cell-free preparations of liver are capable of producing glycogenolysis—that is to say, that they contain the glycogenolytic enzyme, or *glycogenase* (diastase). The conclusion drawn from this was that the vital activity of the hepatic cell is not necessary for the glycogenolytic process, some observers even maintaining that the hepatic cell does not produce the enzyme, but that this, manufactured elsewhere, is carried to it by the blood and lymph.

To follow this question farther, however, we must learn something about the behaviour of glycogenase : how it acts ; how its

activity varies under different conditions ; where it comes from ; what quantitative relationship it bears to the amount of glycogen in the liver ; and, lastly, whether it varies in amount according to the activity of the glycogenic process.

In recent years particular attention has been given to the behaviour of the glycogenolytic (diastatic) enzymes present in the animal body, in the hopes that irregularities in their action might be found to be associated with, or even, perhaps, be the cause of, diabetes. Formerly the glycolytic enzymes received most notice in this regard, but since it has been impossible to demonstrate anything definite with regard to the function of these, even in the normal animal, interest in them has waned in favour of the glycogenolytic, which, as we shall see, are present in considerable concentration in most of the organs and tissues.

Investigation of the action of glycogenase requires, of course, that we separate this enzyme as completely as possible from the other constituents of the tissues in which it is contained. This is, however, by no means an easy thing to do, for, as we shall see, the enzyme is locked up in the protoplasm of the cells, so that these must be disintegrated before it can be set free. It is, for this reason, quite impossible to make any reliable comparison of the ferment strengths of two different tissues by merely mincing or pulverizing these ; we must employ some means to disintegrate the tissue cell and set free the enzymes, and we must also endeavour to obtain a preparation that contains, besides the enzyme in its full strength, as few as possible of the other constituents of the organ or tissue.

There are several ways by which tissue preparations containing large amounts of glycogenase can be made (1). One of these consists in grinding thoroughly the minced tissue along with quartz sand and infusorial earth (Kieselgühr) in a large mortar, then enclosing the putty-like mass in stout sail-cloth, and expressing the intracellular juices by means of a hydraulic press. This is known as the " Buchner process," and the extract obtained from the liver is greyish and usually opalescent ; from more compact organs, such as the muscles, etc., it is much clearer. Another method consists in very thorough maceration of the minced tissue, mixed with quartz sand, in a large mortar, with 0·9 per cent. sodium chloride solution. This method is only possible when soft tissue, such as that of the liver, is used.

A serious objection, which applies to both these methods, is

that the ferment preparations must be used at once, or, at least, cannot be kept without deterioration for more than a day or so, even on ice. It has also been observed that the diastatic action of tissue extracts becomes less after they have stood for some time (2). This is not the case with the diastases in saliva, blood-serum, etc., and it is due to the fact that in extracts of liver prepared at room temperature there are peculiar protein substances which become precipitated on standing, and the precipitate carries down some of the enzyme with it (3).

Permanent preparations can be prepared in two ways. A very favourite method consists in thoroughly mixing the minced tissue with several volumes of alcohol, allowing the mixture to stand a few days, then collecting the precipitate on a filter, washing with alcohol, and drying *in vacuo*. A weighed quantity of the resulting or a watery extract of it is first of all made and a measured powder is then macerated in the solution in which it is to act, volume of this employed. A serious objection to this method is the fact that the glycogenolytic strength of the alcohol precipitate varies according to the time it is left in contact with alcohol (4).

The most recently introduced method, and probably the best for most purposes, consists in spreading the thoroughly pulpified tissue in a thin film on glass plates, and placing these in a strong air current so that the films of tissue quickly dry. The scales are then extracted with cold toluol for several days in a special extraction apparatus, so that all lipoid substances are removed, after which they are easily reduced to a dust-fine powder by means of a mortar and pestle. This powder can then be ground up with water or physiological salt solution, when a considerable proportion goes into solution, the remainder forming a fine suspension, thus making it an easy matter to measure out small quantities of the solution (Wiechowski, 5).

All of these preparations contain, besides glycogenase, several other enzymes, some of which are proteolytic, others lipolytic. In the presence of water, and when the temperature conditions are suitable, these will, of course, become active, with the result that the proteolytic will destroy the other enzymes, whilst the lipolytic will attack any fat that may be present, and, by liberating fatty acid, will cause the solution to assume an acid reaction.

Having isolated the enzyme in as pure a state as possible, we must next proceed to measure *its strength*. There are, in general,

two methods by which this can be done : (1) By measuring the rate of disappearance of the substance acted on (the substrat) ; and (2) by measuring the rate at which the products of the action accumulate. When no intermediate body is formed between the substrat and the final product, and when this latter does not become destroyed by the other enzymes present, we can measure the rate of the reaction by estimating either the amount of substrat left at different periods of time or the amount of decomposition product that has accumulated. But when several intermediate products are formed, and especially when the final product is liable to be attacked by other enzymes, it is evident that, of the above-mentioned methods, only the former can be employed. This is the case with glycogenase ; it produces intermediate substances (dextrines), and although, by itself, it can hydrolyze glycogen and starch only so far as maltose, yet in tissue extracts, as in blood, this maltose becomes converted into dextrose by "maltase," and the dextrose may then undergo glycolysis (6). It is evident, therefore, that the only way to follow the reaction will consist in measuring the amount of substrat remaining after a certain time. In determining the glycogenolytic strength, it has usually been the practice to use starch solutions, for by so doing one can very accurately ascertain the rate at which the starch is becoming converted into dextrine, by using the very delicate reaction that starch gives with iodine. It is much more difficult to determine the same thing in glycogen solutions, partly because dextrines and glycogen give the same brown colour with iodine, and partly because the test is much less delicate.

But, in using starch for this purpose, a possible source of inaccuracy must be borne in mind ; glycogenase and amylase may not be identical. It is now a well-established fact that for each of the disaccharides there is a specific inverting enzyme, and this would at least suggest the possibility that there are likewise specific diastases for the various starches and for glycogen. So far as is known, there is no form of diastase that is not also capable of hydrolyzing glycogen. This is what we should expect when we bear in mind that glycogen in its chemical behaviour is much more like dextrine than amylose ; it is probably a body of lower molecular weight. On the other hand, and for the same reason, it is quite possible that glycogenase might be unable to act on amylose as readily as it does on glycogen. We have

attempted to throw light on this question, but so far have been unsuccessful in obtaining any definite results, largely because of uncertainty regarding the exact chemistry of the starches.

There are, therefore, two methods by which we may determine the activity of enzyme preparations containing glycogenase: (1) By determining the rate of disappearance of starch; and (2) by determining the rate of disappearance of glycogen.

Quantitative methods in which iodine is employed have been developed by Salkowski and by Wohlgemuth. Salkowski, using saliva, mixes a certain volume of this with starch solution, incubates the mixture, and, by means of a pipette, removes from time to time a definite amount to which iodine is added. In this way the time at which the blue reaction disappears is determined. Wohlgemuth adds varying *amounts* of the enzyme preparation to a series of test-tubes, each containing the same quantity of starch solution (5 c.c. of 1 per cent. solution), and, after incubating for a certain time, adds to all simultaneously the same amount of iodine solution (a drop of a $\frac{N}{10}$ solution). The tube which first fails to give the blue colour is noted, and the number of cubic centimetres of the starch solution which 1 c.c. of the enzyme solution could carry to this stage is calculated. This value is called "D," and the duration and temperature of the incubation must be stated along with it.

In our experience these two methods are of equal value. When they are employed for estimating the strength of the diastases in saliva, pancreatic juice, or serum—that is to say, of solutions in which there is no suspended matter—they are accurate enough for all practical purposes. But when it is necessary to determine the diastatic strength of preparations from organs or tissues, they are not reliable, because the suspended particles, which such ferment mixtures contain, absorb the iodine. It is often necessary to add much more iodine than Wohlgemuth recommends, and, even when a sufficiency is added, there is often considerable uncertainty in deciding in which tube the blue colour has just disappeared. With this uncertainty, it is obvious that the method is inapplicable for the detection of small differences in diastatic power (see also 3).

Much more accurate, although consuming more time, is the method in which equal amounts of the enzyme preparations, that are being compared, are added to a series of small (Erlenmeyer) flasks, each containing 10 or 20 c.c. of a 1 or 2 per cent. solution

of glycogen. The flasks are placed in the incubator, and after a definite time* a volume of 60 per cent. potassium hydroxide solution, equal to that of their contents, is added to each, and the glycogen estimated in the usual way. When the enyzme preparations themselves contain varying amounts of glycogen, it is necessary to run a control for each preparation—that is to say, to mix the same amounts of enzyme preparation and glycogen solution, and then immediately destroy the ferment by adding the caustic potash. When none of the enzyme preparations contains any glycogen, or all contain the same amount, one control suffices for the series.

The difference between the amounts of glycogen before and after incubation is a measure of the extent of glycogenolysis, and this difference may be expressed as a percentage of the original amount of glycogen present (percentile glycogenolysis).

Where only slight differences in glycogenolytic strength exist, it is well to employ three flasks for each enzyme preparation. In one of these the glycogen is immediately determined, another is incubated for a short time, and the remaining one for a long time.

Not infrequently it will be found that precipitates develop in the flasks during incubation, especially when relatively large amounts of tissue enzyme preparations are employed. Since these precipitates carry down some glycogen with them, it is necessary to keep the contents of the flask in constant agitation during the incubation.

We come now to the most difficult problem of all—how to measure the relative amounts of the various enzyme preparations used. When such comparisons are being made in the case of fluids—for example, of saliva and blood-serum—there is, of course, no difficulty ; we have simply to measure with a pipette. On the other hand, when we desire to compare the glycogenolytic powers of different organs or tissues, either as between themselves or in contrast with the body fluids, serious difficulties confront us. Suppose, for example, that we desire to compare the glycogenolytic strengths of blood and liver ; we cannot compare an extract of liver, volume for volume, with blood, but we must take

* Since it is often difficult to decide for how long a time the incubation should be allowed to proceed, it is of advantage to mix corresponding amounts of the ferment preparations with starch solutions, and determine, from time to time, the behaviour of each towards iodine by the Salkowski method. This serves as an index of how long to continue incubation.

some criterion of the amount of tissue that is represented in the volume of its extract that we employ. There is no way for doing this accurately, but two arbitrary standards have been used: (1) The nitrogen content; and (2) the ash content. We know what percentage of these the original tissues contain; by ascertaining their percentage also for the extracts, we can determine how much tissue a given amount of the extract represents.

Of these two methods, the nitrogen standard is the more serviceable. Suppose, for example, that we desire to compare the glycogenolytic strength of a series of liver powders, either dried alcohol precipitates or air-dried preparations. We should take 1 gramme of the powder, and grind it thoroughly in a mortar with a little water until a thorough suspension results. The volume having been brought to 10 c.c., 2 c.c. quantities would then be added to the glycogen solutions, as above described, and 2 or 4 c.c. used for a determination of the nitrogen by the Kjeldahl method. After ascertaining the percentile glycogenolysis which has occurred for each preparation, the results would then be calculated in terms of the same amount of nitrogen.*

Before passing on to a consideration of the results, it will be well to indicate briefly the relative value of the above methods for the preparation of the tissue extracts. Although in the earlier observations we did not do this in terms of the nitrogen or ash contents of the extracts, as is recommended above, but merely with quantities of various extracts that should correspond to equal amounts of the tissue used in preparing them, yet the comparisons are instructive, and they demonstrate the necessity of great care in the choice of the method that is to be used. In all cases 20 c.c. of 1 per cent. glycogen were incubated for two or four hours—with the precautions (except shaking) described above—along with 1 gramme of liver, or with an amount of the various enzyme preparations corresponding to 1 gramme.

* In doing this we must bear in mind that we are making the assumption that the extent of glycogenolysis varies directly with the amount of glycogenase present. Should the extent of glycogenolysis vary as the square root of the glycogenase present—that is to say, should it obey the Schutz-Barrisow law, as is the case in peptic digestion (*cf.* 9)—then, of course, the above calculation is unjustified.

Because of this uncertainty, we have, in the majority of our later experiments, chosen quantities of the various enzyme preparations containing the same amounts of nitrogen. Although, by so doing, we do not eliminate the error, yet we can be certain, if the degree of glycogenolysis prove to be equal for the different preparations, that the same amounts of enzyme are present.

No.	Preparation	Percentile Glycogenolysis in Two Hours.	Percentile Glycogenolysis in Four Hours.
1.	Minced liver	16·3	—
2.	Saline extract	9·7	12.3
3.	Buchner extract	29·4	29·2
4.	Air-dried liver powder	29·2	45·9
5.	Alcohol precipitate	34·0	54·8

As judged by the glycogenolysis occurring in two hours, and without any treatment to liberate the enzyme from the liver cells, as in the first two cases, little glycogenolysis occurred. The extracts obtained by grinding air-dried liver saline solution, and by means of the Buchner process, liberated less enzyme than did treatment with alcohol. After incubation for four hours, the air-dried and the alcohol preparations proved themselves by much the strongest. The Buchner extracts generally are not so far behind. In all observations of the above nature made by us, the alcohol preparations have proved to be stronger than any of the others, provided the precipitates had not been allowed to stand under the alcohol for more than a day or so. When allowed to stand for a longer time, the strength of the precipitates falls off, indicating either that the glycogenase has become destroyed or that it has become insoluble. Until the cause of this deterioration is understood, we believe that it is unsafe to employ the alcohol method. It is possible that the greater strength of the alcohol preparations indicates that they are free of some inhibiting substance which is soluble in alcohol and which the other preparations contain.

We are now prepared to consider *the behaviour of the glycogenase present in the liver*. The following questions must be considered :

1. To what extent does the amount of glycogenase in blood-free liver of different animals vary ? Does it bear any relationship to the nutritive condition of the animal ?

2. What quantitative relationship does the hepatic glycogenase bear to the glycogenase present (*a*) in the blood, and (*b*) in the other organs or tissues ?

3. Is the glycogenase produced by the liver cells, or is it merely

transported to them by the blood ? That is to say, is it an intra- or an extra-cellular enzyme ?

4. Are the marked variations in glycogenolysis seen in the liver under various conditions dependent upon an increased amount of glycogenase in this organ ?

5. Supposing there are no variations in the amount of glycogenase, what is it that is responsible for variable degrees of glycogenolysis ?

1. The Relationship of Hepatic Glycogenase to the Nature of the Animal, and to its Nutritive Condition.

Even in organ extracts prepared from animals of the same species and living under the same conditions, there is a considerable variation in glycogenolytic strength. Thus, in observations of this nature in five dogs similarly fed, percentile glycogenolysis, after four hours' incubation, amounted to 25, 25·4, 32, 43, and 58. Similar variations are exhibited by blood-serum. Thus, 1 c.c. of serum from each of six dogs similarly fed produced, during two hours' incubation, 31·9, 25·5, 57·4, 63·0, 64·5, and 100 per cent. glycogenolysis. The strongest sera were noticed to be very opalescent, and the clot firm. These differences are, however, much greater when the preparations are from animals of different species, and they seem to bear some relationship to the nature of the diet ; thus, the liver of the omnivorous dog, cat, or pig yields, in general, a stronger extract than that of the herbivorous rabbit, sheep, or ox. Indeed, it is said that alcohol preparations of the liver of the sheep or rabbit have no glycogenolytic action (11).

There is, apparently, no content relationship between the amounts of glycogen and glycogenase present in the liver ; thus, we have found that a Buchner extract of the liver of a starved dog exhibited, in four hours, 44 per cent. glycogenolysis, and an equal volume of one from an excessively fed dog only 30 per cent. Bang, etc., also found slightly more glycogenase in the livers of starved rabbits than in those of rabbits that were well fed (12). But in neither of these observations were the results calculated according to the amounts of nitrogen or ash present in the extracts, and it is therefore probable that the differences observed were due to greater dilution of the extracts prepared from the

well-fed animals by the large amounts of glycogen and fat present in the liver.

The results are, however, sufficient to show that the variations in glycogenase content do not run parallel with those of glycogen. The entire want of parallelism between the amounts of glycogenase and glycogen is well illustrated by Maclean's observations. Confirmatory results have been obtained from the examination of blood-serum (1, 10, 13, 22).

2. The Quantitative Relationship between the Glycogenase of the Liver, the Blood, and the other Organs and Tissues.

The liver is not the only organ which contains glycogenase. The blood, as we have seen, contains a large amount of it, and so do blood-free extracts prepared from the heart, intestines, muscles, and kidneys. The question naturally arises as to the source in the body of the glycogenase ; is it produced in one place, and transported elsewhere in the body by the blood and lymph ? or, is it locally produced in each organ or tissue that contains it ? We are accustomed to think of specific enzymes as being produced in some gland whose cells are specialized for this purpose rather than of their being products of the activity of many different types of cell ; but although this localization in enzyme production is very marked in the digestive system, we have no very definite evidence that it obtains for those enzymes that are widely scattered in the animal body. These may be produced locally in the various tissues in which they are found present.

The two best known of these enzymes are erepsin and glycogenase ; both are widely distributed in the animal body, but there is a fundamental difference between them, for erepsin is almost absent from the blood, which, however, contains large quantities of glycogenase. The belief at the present time is that erepsin is locally produced in the cells in which it is found ; it stays and acts at the site of its production, and is therefore to be considered as an intracellular enzyme. On the other hand, there has been a tendency to consider glycogenase as being produced as a special function of some organ, from which it is transported by the blood to wherever else it may be found present. It does not, therefore, necessarily unfold its action only where

produced, but may be carried as an extracellular enzeme by the blood to the places where glycogen exists. Attention must be called to the fact that between this circulating glycogenase and the glycogen on which it is to act there exists a barrier of living protoplasm, and that the permeability of this layer of protoplasm may be subject to changes that are under the control of the nervous system, etc.

By a comparison of the amount of glycogenase found present in the various organs and tissues, some idea can be formed of its possible source, but we must not expect that we are to be able to draw final conclusions from the results of such comparisons. Pick (14), for example, finding that the liver freed from blood contained more glycogenase than an equal volume of blood, concluded that this enzyme must be produced in the liver, whence some of it overflows, as it were, into the blood. But such a type of argument postulates that enzymes diffuse more or less passively out of the cells, and, of course, there is no justification for such a view. Even if it were the case that the liver contained somewhat more glycogenase than the blood-serum, it would not necessarily follow that the glycogenase had been produced in the liver ; it might have been absorbed from the blood, and stored there on account of some attraction for glycogenase on the part of the liver cell. On the other hand, should a *very large* excess of glycogenase be found in one organ, as compared with all the others in the body, including the blood, it would be strong, although perhaps not final, evidence of production in this organ.

Looking at this question from another point of view, we must remember that there need not be any excess of an enzyme demonstrable in extracts of that gland where it is manufactured. A saline extract of the stomach possesses, for example, but faint peptic powers, because the extract contains, not pepsin, but its precursor, pepsinogen, which must be activated by means of acid. So with the cells that produce glycogenase ; they may not contain this as such, but as a pro-enzyme, and we may not, so far, have been successful in discovering the activator.

Taking all these facts into consideration, it is evident that although comparisons of glycogenase concentration must be the first step in our search for the seat of production of this enzyme, it cannot be expected that it will finally settle the question. Comparisons of this nature have been made by R. G. Pearce and the author in the case of blood and of the muscles freed from blood,

liver, intestine, kidney, and pancreas. Saline and Buchner extracts were employed.

The following condensed Table (I.) illustrates the results :

No. of Experiment.	Organ.	Percentile Glycogenolysis.	Duration of Incubation.	Remarks.
J	Pancreas	100·0	2 hours	1 : 10 saline extract.
	Liver	92·5	,,	Buchner extracts.
	Serum	39·5	,,	
	Muscle	18·0	,,	
	Intestine	9·2	,,	
M	Pancreas	100·0	4 hours	1 : 8 saline extract.
	Liver	88·6	,,	Buchner extracts.
	Serum	83·3	,,	
	Kidney	48·9	,,	
	Muscle	0·0	,,	
I	Pancreas	100·0	1 hour	1 : 50 saline.
	Serum	30·8	4 hours	1 : 1 saline extracts.
	Submaxillary gland	11·7	,,	
	Liver	9·4	,,	
	Muscle	6·0	,,	
	Heart	4·7	,,	

With regard to their glycogenolytic powers, we may divide the organs and tissues investigated into three groups. The pancreas forms a group by itself, and is many, many times stronger than the others ; next, and of about equal strength, the blood and liver ; and, of considerably less strength, the muscles, kidneys, intestine, and heart.

The results are not entirely in harmony with those of other investigators. Leaving out of consideration for the moment the relative strength of blood and liver, Wohlgemuth and Benzur have found that the kidneys and muscles of the rabbit contain more glycogenase than the liver. We have already seen that compared with other animals the liver of the rabbit contains very little glycogenase. There is evidently, then, some difference in distribution of glycogenase according to the kind of animal.

Maclean (10), using alcohol powders and determining the diastatic activity from the amount of sugar formed after incubation with starch solutions in the presence of toluol for eighteen to twenty hours,* found the results very variable even when preparations from corresponding tissues of animals of the same species

* The objections to this technique have been given on p. 138.

were compared. Thus, in the cat, the rabbit, the dog, and in one sheep, the kidneys gave the strongest preparation, the liver yielding one that was much weaker. In most animals the muscles gave the feeblest preparation of all. Sometimes the lungs were the strongest of all. In one case only (the sheep) did the liver contain the largest amount of glycogenase. It must be pointed out that the blood was removed from the tissues in Maclean's experiment by washing small pieces of tissues in saline solution. This is probably not thorough enough.

The most interesting comparison is that between the blood and liver, for it is in the liver that there are the largest stores of glycogen for the glycogenase to act upon. And, although they are by no means conclusive, as has been pointed out, such comparisons furnish us with a clue as to whether the glycogenase of the liver cell is manufactured there or is derived from the blood. In order to make this comparison, however, it becomes necessary to collect more evidence than is afforded above. This we have done in further experiments, with the results given in the following Table :

No. of Experiment.	Animal.	Percentile Glycogenolysis in—		Time of Incubation.	Remarks.
		Serum.	Liver.		
D	Dog	100·0	7·6	16½ hours	—
E	,,	60·0	55·1*	16 ,,	* Blood not washed out of liver.
F	,,	100·0	31·9-45·3†	3½ ,,	† Reaction varied.
O	,,	37·7	100·0	1¼ ,,	Starved dog.
P (1)	,,	31·9	30·0	2 ,,	Overfed dog.
	,,	100·0	58·2	5 ,,	—
P (2)	,,	70·5	52·5	2 ,,	—
R	,,	57·4	7·2	2 ,,	—
S (1)	,,	100 (?)	44·1	2 ,,	Starved dog.
S (2)	,,	100 (?)	28·4	2 ,,	Ordinarily fed dog.
T	,,	63·8	42·8	3 ,,	—
W	,,	63·0‡	10·5	2 ,,	‡ Exact time of incubation uncertain.
Z	,,	64·5	17·9	2 ,,	—
	Lamb	19·6	5·7	4 ,,	—
	Pig	71·0	39·0	4 ,,	—
U	Rabbit	72·3§	34·2§	4 ,,	§ After standing four days.
	,,	38·5	12·2	2 ,,	—

In the observations on the dog, the serum was distinctly the stronger in eleven, and the liver in one. In this case, however,

the animal had refused food for several days before the experiment, and it was undoubtedly ill. In the other animals investigated the serum was always stronger than the liver.

Although the majority of the investigators in this field (*cf.* 16) have obtained similar results to the above, this has not universally been the case ; thus, Pick and Mendel and Saiki (17) found the liver stronger than the serum, which, however, is probably to be accounted for by their having employed the alcohol method for isolation of the ferment (p. 138). Contrary results were also obtained by Pugliese and Domenichini, but, since these workers measured the rate of reaction by the amount of sugar which was produced, their results cannot be accepted (p. 139). So far we may conclude that the serum possesses a greater glycogenolytic power than an amount of liver extract corresponding to an equal volume of liver tissue. This does not, however, necessarily imply that the serum contains a greater amount of glycogenase than the liver ; the observed difference may be due to a loss of enzyme incurred in the preparation of the extracts—that is to say, a large part of the glycogenase present in intact liver might become destroyed during the preparation of the extracts, whereas that in blood remained of full strength. This weakening of the glycogenolytic strength might result from the presence of acids produced in the liver by the autolytic process, or because a part of the glycogenase had become absorbed by the infusorial earth, which is used in preparing extracts by the Buchner process.

It is, of course, well known that the activity of diastatic enzymes is markedly influenced by the reaction of the fluid in which they are acting. The experiments demonstrating this have been performed mainly with starch solutions mixed with saliva, pancreatic juice, or malt diastase (18). Briefly stated, it has been found that a very low degree of acidity accelerates the hydrolysis of starch, whereas a higher degree depresses it ; there is, in other words, an optimum acidity. When, therefore, the reaction of the mixture of diastase and starch solution to start with is neutral, or only faintly alkaline, the addition of a certain amount of acid will accelerate the hydrolysis, but the same amount of acid, added to a mixture of which the initial reaction is faintly acid, will depress the hydrolysis. The addition of even the minutest trace of alkali to a mixture of which the original reaction is faintly alkaline, or exactly neutral,

causes distinct depression in the diastatic power ; but when the alkali is added to a mixture of which the reaction is acid, its effect will, of course, depend on the degree of acidity. If this be so high that it has overstepped the optimum of acidity, the alkali will cause acceleration ; if it be at or below the optimum, the alkali will cause retardation.

The following Table illustrates these points in the case of blood-serum and liver extracts :

No. of Experiment.	Degree of Acidity or Alkalinity.	Percentile Glycogenolysis in—		Remarks.
		Serum.	Liver.	
T	Original reaction	63·8	42·8	One c.c. serum or extract + 20 c.c. 1 per cent. glycogen solution incubated 3 hours.
	0·0078 per cent. acetic acid	77·4	23·3	
	0·0224 ,, ,,	66·0	27·0	
	0·0390 ,, ,,	—	5·6	
T	0·2 c.c. 1 per cent. Na_2CO_3	28·4	—	Do.
	0·6 ,, ,,	0·0	—	
	1·0 ,, ,,	0·0	—	
A	Original reaction	26·9	29·4	Same in experiment T, except that 2 c.c. liver extract used, and incubation continued for four hours.
	0·2 c.c., 1 per cent. Na_2CO_3	19·6	28·1	
	0·4 ,, ,,	6·6	23·6	
	0·6 ,, ,,	9·6	24·9	
	1·0 ,, ,,	—	9·0	

It is seen that the addition of a small amount of acid accelerates the glycogenolytic action of the serum, but depresses that of the liver. Alkali addition, on the other hand, depresses the glycogenolytic action in serum, but has little effect on that of the liver extracts until a considerable excess has been added.

During the autolytic processes which take place in the liver cell after death, acids (sarcolactic) are produced (Magnus Levy), and, besides these, the Buchner extracts must contain lipases which, by acting on any neutral fat therein present, will liberate fat-acid during the incubation. As a matter of fact, when any large proportion of the Buchner extract has to be employed, the incubation mixtures do increase in acidity towards litmus. The possibility has therefore to be considered that this acidity might be so great as to depress the glycogenolytic powers of the extracts to such a degree that they appear weaker than the serum.

By carefully neutralizing the incubation mixtures, not only

at the start of the incubation, but at half-hour intervals during it, we have been able to show that such is not the case; the careful neutralization of the liver extracts does not make them nearly so strong in glycogenolytic power as the blood.*

The possibility that some of the glycogenase might become removed from the liver extracts by becoming absorbed by the infusorial earth requires some notice, because Hedin has found that *a*-protease, an enzyme in the spleen, is absorbed by this substance. That such an absorption of glycogenase does not occur, however, was shown in control experiments of two types; in one the serum was treated with infusorial earth in the same manner as the liver, and in the other the liver extract was prepared without the addition of any of the earth. Finally, to the above observations it might be objected that blood-plasma instead of blood-serum should have been employed. Such an objection would be sound if anything happened to the glycogenase during the process of clotting; *e.g.*, it might become increased in amount by the leucocyte disintegration, or it might become lessened in amount by being absorbed by the fibrin, for Vernon has shown that malt diastase is absorbed by this substance. We have accordingly compared serum and (oxalate) plasma, as regards their glycogenolytic powers, and have found them to be identically the same. Wohlgemuth (13) obtained the same results by the use of starch solutions.

In practically all of the experiments referred to above the basis of comparison between liver and blood is that of volume measurement. Admittedly, the comparison is a faulty one, but inasmuch as care has always been taken to use for the comparisons an amount of liver extract considerably in excess of that which could come from a volume of liver equal to that of the blood, any error in the above results must be to the favour of the liver. It is certain that in the majority of animals, if not in all, *there is more glycogenase in the blood than in an equal volume of liver.*

The higher concentration of glycogenase in the blood than in

* For the determination of the reaction in these experiments, litmus was the indicator used, so that the observations must be considered as approximate only. A much better way of deciding the matter would have been to allow the digestions to proceed in the presence of alkaline sodium phosphate, or of a phosphate mixture of the same $H^{\cdot}$ ion concentration as blood-serum.

the blood-free liver renders it necessary for us to inquire whether that which is present in the latter may not be derived from blood or lymph which has not been entirely removed from the viscus by the washing process. Such is the view of Bial (19). In order to test this possibility we have performed the following experiment: An anæthetized dog was bled to death, and, after removal of a portion of a small lobe, the liver was perfused with 0·9 per cent. saline until the washings just became untinted with blood, when a second small lobe was removed. The remainder of the liver was then perfused with several decalitres of saline, and was actively massaged so that all traces of lymph might be removed from it. Saline extracts of corresponding strengths were carefully prepared from the three portions of liver, and the following percentile glycogenolysis was obtained with each:

The extract of the unwashed liver produced	..	41·5 per cent. glycogenolysis.
The extract of the first washed liver produced	27·4	,, ,,
The extract of the second washed liver produced	28·6	,, ,,

The prolonged washing with saline has removed no more glycogenase from the viscus than the moderate washing, which makes it highly improbable that retained lymph can be held accountable for the glycogenolytic action of blood-free liver. Evidently, the glycogenase is within the liver cells ; and it would appear, from the results of the last-mentioned experiment, to be pretty firmly fixed there, either because it cannot diffuse out through the cell wall, or because, like erepsin, it is united by some chemical bond to the protoplasm of the cell. We tried in one experiment to dislodge some of the glycogenase by tying the portal vein of a liver that had already been washed blood-free with saline, to a water faucet, and allowing water to perfuse through the viscus for about an hour, but the glycogenolytic power of an extract prepared from this portion of the liver was only slightly inferior to that of an extract prepared from the same liver just after removal of the blood (the former gave 67 per cent. and the latter 71 per cent. glycogenolysis in five hours' incubation).

But although glycogenase is a constituent of the liver cell, this does not imply that it is produced here ; it may be transported hither by the blood. As was explained above, it has been

mainly for the purpose of deciding whether this is the case that the distribution of glycogenase in the animal body has been studied. We have discussed the value of such results in this connection, and have shown that the conclusion which we can draw from them is that if a *very large excess* of glycogenase were found in one viscus over all the others, this might be provisionally considered as the seat of glycogenase production. We have found such an excess of glycogenase in the pancreas, the next largest amount being present in the blood. The slope of glycogenase pressure is, as it were, from pancreas to blood, and then from blood to the various organs and tissues. Is the pancreas, then, the seat of manufacture of all the glycogenase present in the animal body? If it is, we should be able to find, under certain conditions, at least, that there is more glycogenase in the blood of the pancreatic veins or in the lymph of the thoracic duct than in the blood or lymph coming from other regions of the body. There is now abundance of evidence, collected partly by ourselves and partly by other investigators, to show that such is not the case, at least, in so far as the blood of the pancreatic veins is concerned; there is as much glycogenase in the blood of the systemic circulation as in that of the pancreatic veins. Of course, one must bear in mind that our method for measuring glycogenolytic strength may not be refined enough to detect the very small amount of glycogenase which it would be necessary for the pancreas to add to the blood in order to keep the supply of glycogenase up to the necessary level. Many false conclusions have been drawn in physiology from the results of observations of this type—that is to say, when attempts are made to decide, by comparison of the inflowing and outflowing blood, whether a given organ or tissue adds something to, or removes something from, the blood. When positive results are obtained, there can be no doubt about the conclusion, but when these are negative, it need not indicate that the organ has failed to produce a change, but simply that this change is so small that we cannot detect it; for this it may be, and yet the total change in twenty-four hours be enormous.

Ehrmann and Wohlgemuth (20) did not find the blood of the pancreatic or the portal vein to be any stronger in amylase than that of the femoral artery; but for such slight differences as would probably exist, did any exist at all, the colorimetric method employed by these authors is scarcely accurate enough.

R. G. Pearce and the author have repeated the observations by the use of the more accurate methods described above, and they have considerably extended them by collecting the blood and lymph under experimental conditions which are known to be the cause of a hyperglycogenolysis. The condition chosen for this purpose was stimulation of the great splanchnic nerve (see p. 61). The lymph was collected from the thoracic duct (21).

The Table on p. 155 gives the results.

In Experiments 4 and 8 there was no evidence of increase of glycogenase in either blood or lymph *during* stimulation of the splanchnic nerve. There was a slight increase in the ferment contained in the femoral blood *after* the stimulation in Experiments 8 and 9, but this did not occur until from sixty to eighty minutes after the stimulation had been removed, and it is very doubtful whether the stimulation can be held responsible for it. In Experiment 3 there was a slight increase, both in the blood of the pancreatic vein and in lymph, immediately following the stimulation. In Experiment 6 there was an increase in the lymph and in the femoral artery blood during the stimulation. The only experiment, therefore, in which splanchnic stimulation certainly caused a rise in glycogenase was No. 6. The increase occurred primarily in the lymph and secondarily in the systemic blood ; it fell off in both after the stimulation was removed.

It would appear that an increase in the glycogenase content of the lymph may sometimes occur when the liver is excited to increased glycogenolysis by stimulation of the splanchnic nerve, but this does not imply that the pancreas has produced more glycogenase, for it must be borne in mind that the thoracic lymph is derived in large part from the liver and that only a small fraction of it comes from the pancreas.

From such experiments, then, there is nothing to indicate that glycogenase is directly secreted into the blood by the pancreas, although there is some evidence that it may be secreted by some one of the abdominal viscera into the lymph. It would be rash to draw a final conclusion from the result of a single experiment, but, as explained above, one positive result is of much more value in the present connection than several that are negative.

The diastatic activity of lymph collected from different lymph vessels, in the cat and dog, has been compared with that of blood-serum by Carlson and Luckhardt (22). The serum

GLYCOGENASE IN THE BLOOD AND LYMPH AS AFFECTED BY STIMULATION OF THE SPLANCHNIC NERVE.

Number of Experiment.	Nature and Amount of Fluid Used.	Experimental Condition.	Glycogen (Dextrose) which Disappeared in Same Time.	Remarks.
3.	Femoral artery	Before stimulating great splanchnic nerve	—	Incubated 3 (?) hours. 1 c.c. serum. Starch test gave dextrines first with *.
	,, ,,	After 15 minutes' stimulation	0·073	
	Pancreatic duodenal vein	Before stimulation	0·068	
	,, ,, ,, *	After 15 minutes' stimulation	0·076	
	Lymph	Before stimulation	0·067	
	,,	After 15 minutes' stimulation	0·075	
4.	Femoral artery	Before stimulating great splanchnic nerve	0·108	Incubated 3 hours. 1 c.c. serum. Starch test gave dextrines in 60 minutes with all.
	,, ,,	During 1 hour's stimulation	0·108	
	,, ,,	30 minutes after stimulating off	0·103	
	Pancreatic duodenal vein	Before stimulation	0·103	
	,, ,, ,,	During 1 hour's stimulation	0·103	
	,, ,, ,,	30 minutes after stimulating off	0·105	
6.	Femoral artery	Before stimulating great splanchnic nerve	0·074	Incubated 4½ hours. 1 c.c. serum in each case. Starch test gave dextrines first with *.
	,, ,,	During 30 minutes' stimulation	0·101	
	,, ,,	45 minutes after stimulating off	0·079	
	Pancreatic duodenal vein	Before stimulating	0·081	
	,, ,, ,,	During 30 minutes' stimulation	0·082	
	,, ,, ,,	45 minutes after stimulating off	0·090	
	Lymph	Before stimulation	0·088	2 c.c. serum incubated 3½ hours.
	,, *	During 50 minutes' stimulation	0·115	
	,,	45 minutes after stimulating off	0·101	
8.	Femoral artery*	Before stimulation	0·065	Incubated 1 hour. 1 c.c. serum. Starch test gave dextrines first with * * *.
	,, ,, *	During 20 minutes' stimulation	0·065	
	,, ,, *	80 minutes after stimulating off	0·076	
	Lymph	Before stimulation	0·051	
	,,	During 20 minutes' stimulation	0·047	
	,,	60 minutes after stimulating off	0·045	
9.	Femoral artery	Before stimulation	0·075	Incubated ½ hour. 5 c.c. serum.
	,, ,,	During 80 minutes' stimulation	0·082	
	,, ,,	60 minutes after stimulating off	0·093	
	Pancreatic duodenal vein	Before stimulation	—	
	,, ,, ,,	During 80 minutes' stimulation	0·082	
	,, ,, ,,	60 minutes after stimulating off	0·080	

was the strongest, then the thoracic lymph ; the neck lymph and the pericardial fluid were the feeblest. Increasing the lymph flow by injecting lymphagogues only sometimes caused the diastatic power to become greater. Röhmann and Bial (23) also found the thoracic lymph of feebler diastatic power than the blood-serum.

If the pancreas be the source of the tissue diastases these would be expected to change in amount when the gland is removed or is stimulated to increased activity. Their behaviour after pancreatectomy has been investigated by Bainbridge and Beddard, Carlson and Luckhardt, Schlesinger, Wohlgemuth, and by Otten and Galloway (34). The first and second groups of authors found that pancreatectomy in cats did not affect the concentration of the diastases of the blood or lymph. Schlesinger, on the other hand, in two dogs and one cat, came to the opposite conclusion—namely, that after pancreatectomy there is a complete disappearance of diastase from the blood. Wohlgemuth could not confirm this result, but found in ten dogs that complete pancreatectomy caused only a temporary decline in the diastatic power of the blood. Otten and Galloway observed the amylolytic power of serum of blood collected from the ear or from a leg vein at intervals of about three days following pancreatectomy in dogs. Three of the animals survived the operation for nearly three weeks. It was found that, on the day following the operation, the diastase was very markedly decreased as compared with that found present in a sample of blood drawn prior to the operation. It either remained at this low level for a day or so longer or it began immediately to mount again, until, in a little short of a week after the operation, it had found a level at which it remained constant until the death of the animal. A mock operation, in which the abdomen was opened and the duodenum, etc., manipulated, caused but a slight and transient decline in the blood diastases. Taking these results as a whole we may conclude that a sharp decline in the circulating glycogenase immediately follows removal of the pancreas, but that a certain recovery soon becomes established, possibly indicating that some other organ than the pancreas is producing the glycogenase.

The effect of increased activity of the pancreas has been studied by Wohlgemuth (25), who examined the diastatic power of blood from the leg veins in dogs after injecting secretin or administering

hydrochloric acid by the stomach; but no change was observed. In other experiments the pancreatic ducts were ligated, with the result that a marked increase in diastatic power of the blood occurred, and persisted for about a week. This effect was much less pronounced when only one duct was ligated.

The balance of evidence stands in favour of the view that the pancreas is the seat of production of the glycogenase of the blood and lymph; it remains uncertain, however, whether the glycogenase found present in the various organs and tissues is appropriated from the blood or is locally produced. When the pancreas is absent, there is evidence that the function of producing glycogenase may be assumed by other tissues.*

Notwithstanding the absence of any apparent relationship between the glycogen content and the glycogenolytic activity of extracts of the different organs and tissues, it is undoubtedly as a result of the action of glycogenase that sugar is produced from glycogen in the animal body. Knowing that the most marked variations in this sugar production occur in the liver, it becomes of great importance to find out whether they are dependent upon changes in the amount of glycogenase in the liver cells. The amount of enzyme in the secretions of the digestive glands is more or less proportional to the amount (and nature) of the substance that has to be digested. Is this also the case in connection with the glycogenic function? The digestive glands and the glycogenolytic function of the liver are both subject to nerve control; the submaxillary gland secretes an increased amount of very active saliva when the sympathetic nerve-supply is stimulated, and, under the same condition, the liver secretes more sugar, which may indicate that the nerve stimulation has caused an increase in the amount of glycogenase. Although many have accepted this view, the experimental evidence which has been offered in support of it (27) can easily be shown to be fallacious.

If an increase in glycogenase does really occur during hyperglycogenolysis, it should be evident when comparisons are made of the glycogenolytic strength of extracts of liver removed from the animal before and during stimulation of the splanchnic

* It has been suggested that the diastase produced by the pancreas may find its way into the blood by being absorbed from the lumen of the intestine (Schlesinger, 26).

nerve. Such comparisons have been made by R. G. Pearce and the author (28). By choosing for the investigations sufficiently large dogs, these workers were able to avoid the error which is always incurred by making comparisons between results obtained on different animals—that is to say, between one set of animals considered as normals and another set in which hyperglycogenolysis has been experimentally produced.

By the method described in the paper referred to, quantities of blood were removed at frequent intervals from the vena cava opposite the liver, and the sugar content of these was determined in order to make certain that hyperglycogenolysis had really been produced by the stimulation. In many of the experiments the glycogenolytic power of the serum was also determined, in order to see whether any glycogenase became discharged into the blood during the increased glycogenolysis.

After ligation of each mass three portions of liver were removed—(1) before, (2) during, and (3) after stimulation of the splanchnic nerve. After removing the blood, extracts were prepared from each of these by exactly the same technique, and their glycogenolytic powers measured in the manner described above. The results were evaluated in terms of the nitrogen content of the different extracts. To maintain the reaction as constant as possible, a small amount of phosphate mixture was added to each incubation flask. Besides the phosphates, the incubation solutions also contained isotonic quantities of sodium chloride so that the Cl ion, which has a marked influence on diastatic activity (see 8), might be constant in amount.

In the first series of observations the enzyme preparations were made by the Wiechowski method. It was first of all observed in two dogs that no change occurs in the glycogenolytic power, either of the blood of the vena cava or of the liver itself, when the animal is kept for an hour under ether anæsthesia. As already stated, it is usually the case that the reducing substance in the blood gradually declines under these conditions in sugar-fed animals (see p. 188). In all but one of seven experiments, in which the splanchnic nerve was stimulated, there was evidence of hyperglycogenolysis, as revealed by the amount of reducing substance in the vena cava blood. In five of these seven, the liver preparations before, during, and following the stimulation of the splanchnic nerve exhibited equal glycogenolytic powers, but in two of them an undoubted increase in this

was observed. In four of the experiments the glycogenolytic power of the blood of the vena cava was also measured; it remained unchanged, except in one case, in which it became higher, after the removal of the stimulation.

The two positive results in this first series of observations might be taken to indicate that, in these cases at least, more glycogenase had been produced in the liver as a result of stimulation of the splanchnic nerve. Indeed, they might be considered as sufficient evidence that such an increase *always* occurs under these conditions, but that the increase is undetectable in the majority of cases because of unsuitable periods of time being chosen for the incubation of the mixtures. For when, as in these investigations, the duration of the incubation periods for each of the different liver preparations is made the same in any given experiment, slight differences in glycogenolytic power might not reveal themselves, because the glycogenolytic process obeys the logarithmic law (see p. 162), and consequently the differences, if any exist, will be more marked early in the incubation period than later. It became necessary, therefore, to repeat the experiments, using different periods of incubation. It was further decided not to depend on one method alone for making the enzyme preparations, but to employ several. Those chosen were extraction with isotonic saline solution and the Buchner process, usually carried out at a temperature near the freezing-point.

The method for removing blood from the liver was also improved, by taking pieces that were large enough to permit of perfusion with isotonic saline. The results are given in condensed form in the Table on p. 160.

In two of these experiments the blood of the vena cava was also examined, and found to be of the same glycogenolytic power, before and during the stimulation.

After the elimination of all sources of error, it is clear from these results that *increased glycogenolytic activity of the liver, brought about by stimulation of the splanchnic nerve, is not accompanied by any change in the amount of glycogenase*, either in the liver* itself or in the blood issuing from it. It will be remembered that there is also, as a rule, no change in the glycogenolytic power of the lymph of the thoracic duct. We must assume that

* It is probable that the slight increases found in the earlier series were due to imperfect removal of blood, for the portions of liver were small, and could not be perfused.

it is because of alterations in the conditions under which glycogenase, constant in amount, acts, that changes occur in the intensity of the glycogenolytic process.

GLYCOGENASE CONTENT OF LIVER BEFORE AND DURING STIMULATION OF THE SPLANCHNIC NERVE.

No. of Experiment.	Mode of Preparation of Liver.	Time of Incubation.	Percentile Glycogenolysis of Liver for Equal Amounts of Nitrogen.	
			Before Stimulation.	During Stimulation.
		Minutes.		
34	Air-dried powder of washed liver	—	67·0	—
			65·4	65·7
35	Saline extract of washed liver	—	40·9	40·1
39	Buchner extract of perfused liver	—	33·6	28·3
40	Do. Do.	90	13·5	13·4
		180	27·0	30·0
		420	48·5	42·5
60	Buchner extract made at low temperature, from liver perfused with ice-cold saline	120	32·1	31·8
		210	49·2	45·2
		300	63·9	59·0
63	Saline extract made at low temperature ,from liver perfused with ice-cold saline	90	18·7	25·6
		180	—	44·2
		300	61·2	61·0
64	Do. Do.	90	21·8	20·1
		180	42·77	42·5
		300	52·2	51·7

Before we proceed to discuss the possible nature of these changes in the liver, we shall turn our attention for a short time to another condition in which there is excessive glycogenolysis—namely, to *post-mortem* glycogenolysis. A knowledge of the conditions which control this process, more especially the conditions of production of glycogenase, will aid us in arriving at the cause of the variations which exist in the case of the ante-mortem process. In post-mortem glycogenolysis we are provided with a much simpler condition than that which pertains when the liver is in the body; in the former, the stimulus must arise in the liver itself, whereas in the latter case, some enzyme, or other substance, carried to the liver in the blood, may be the agency which is responsible for increased glycogenolysis, when such occurs. It therefore becomes of great interest for us to see whether post-mortem glycogenolysis is due to a production of glycogenase or to the development of some condition which facilitates the action of an unchanging amount of this enzyme.

To investigate these possibilities, however, we must first of all learn something about the nature of the post-mortem process itself. We must find out at what period it sets in, with what speed it assumes its maximal intensity, and whether, after attaining this maximum, it remains constant or immemediately begins to decline. By ascertaining these facts, we shall not be in a position to conclude whether post-mortem glycogenolysis is due to the accumulation of new enzyme in the viscus, or to increased enzymic activity resulting from changes in the condition of action of a constant amount of enzyme, but we shall be able to judge at what stages in the process it will be of value for us to extract the ferment and study its action on glycogen solutions, in order to see whether it has changed in amount.

It is a very difficult matter indeed to determine, after the cessation of the circulation, the exact time at which the glycogen begins to disappear with abnormal rapidity, because the time-intervals between which portions of liver must be removed for analysis are very short and experimental errors are great. Another serious difficulty arises from the fact that central portions of liver cannot be removed before the death of the animal, because glycogenolysis occurs during anæsthesia.

In two successful experiments on rabbits, however, we were able to show that the process is well-established within twenty minutes after death (29). Pavy (30) had previously concluded that the process is established in a few minutes after death.

More accurate information, might be obtained by observing when the sugar output from the hepatic veins increases after temporarily placing a clamp on the portal vein (see p. 171). Such observations, however, have not been made with this point in view.

Having once become established, the process seems to develop to its maximum very rapidly, and then to proceed at a fairly constant intensity for some considerable time. These facts are best elicited by a study of the *velocity constant*. This value makes allowance for certain changes occurring in a fermentation mixture during the progress of enzymic action, which must be taken into account before any conclusions can be

drawn as to whether the enzyme has altered its activity. One of these changes is the gradual diminution in the amount of substance on which the enzyme can act—the "substrat," as it is called. Were no allowance made for this, it is obvious that, at an early stage in the reaction, a given amount of enzyme would be capable of producing a greater absolute change than at a later stage, when only a small amount of substrat is available. We have allowed for this, to a certain extent, in previous experiments, by calculating the amount of glycogen decomposed in a given period of time as a percentage of the amount of glycogen present at the beginning of that period.

For our present purpose, however, we must calculate more closely. We must use, not the amount of undecomposed glycogen present at the *beginning* of a time period, but the amount present *at every moment* during the change. The following equation has been evolved to allow for this : $\frac{1}{t}$ log. nat. $\frac{c^*}{c_1}$ where C represents the amount of substrat to start with and C_1 the amount remaining at the various periods t. By employing such an equation, it is found that when the agency (enzyme) which is influencing the speed of the reaction remains unchanged, and no disturbing influences, such as the reaction of the mixture, undergo variation, a constant value is obtained. Such is the case, for example, in the hydrolysis of cane-sugar to invert-sugar, or of methyl-acetate to methyl-alcohol and acetic acid by means of a mineral acid. By employing this equation for the calculation of the rate of disappearance of glycogen from the liver, we are able to see whether the glycogenolytic enzyme is changing its activity at different stages in the process. The equation does not apply, however, to cases in which a large excess of substrat is present in comparison with the amount of enzyme, for then the *absolute* amounts of substrat decomposed in equal periods of time will be the same. It can be imagined that, under such conditions, all of the enzyme is continually occupied with as much of the substrat as it can act on, whereas, when the substrat is less in amount, some of the enzyme is un-

* This equation is obtained by integration from the equation representing the progress of a monomolecular reaction : $\frac{dx}{dt}=C\ (A-x)$. Where A and x stand respectively for the substrat and the products of the reaction in the time t, and C is a constant.

occupied even at the beginning of the process, and the proportion of this unoccupied enzyme progressively increases as the reaction proceeds. The above equation allows for this. We do not as yet know when the one condition gives place to the other in the glycogenolytic process, but it would appear that there is seldom, if ever, so much glycogen present in the liver that the logarithmic equation does not apply.

We have determined the velocity constant for post-mortem glycogenolysis in the case of two livers containing average amounts of glycogen, and removed from dogs killed as rapidly as possible by ether and hæmorrhage. The livers were kept at body temperature in the incubator, and portions were removed for the estimation of glycogen at the end of every hour. The following are the results :

No. of Experiment.	t (Hours).	C_1 (Grammes Glycogen in 100 Grammes Liver).	$\frac{1}{t}$ Log. Nat. $\frac{C}{C_1}$.
1	0	(C. 4·345)	—
	1	3·826	(0·5540)
	2	3·620	0·0396
	3	3·480	0·0321
	4	3·100	0·0366
	5	2·924	0·0343
	6	2·615	0·0367
	7	2·265	0·0404
	8	2·015	0·0417
	9	1·800	0·0425
2	1	4·165	0·0449
	2	3·866	0·0368
	3	3·576	0·0370
	4	3·230	0·0388
	5	3·186	0·0323
	6	3·033	0·0304

In both cases the enzymic action was more rapid at the beginning of the process, and then proceeded at an almost uniform velocity till about the seventh hour (in the first experiment) when it began to increase again, probably because of commencing putrefaction.

The conclusion which we can draw is that there is no progressive increase in the dead liver of the conditions which are responsible for post-mortem glycogenolysis. These become established early, and then remain more or less constant.

The only other investigation of a similar nature that we are aware of is by Taylor (31), who determined the velocity constant for the disappearance of glycogen in the mixed livers of several clams kept under toluol at an equitable low temperature (12·4° C.) in a deep well. He found it to decrease after several hours, and concluded that the glycogenolytic enzyme suffered progressive inactivation, and this he ascribed to its gradual destruction by hydrolysis.

These results throw no light on the causation of the glycogenolytic process. It may be either that glycogenolytic enzyme becomes produced, or that this does not increase conditions favourable to its action and develop. To determine which of these alternative causes is the real one, we must separate, as far as possible, the enzyme from the liver and study its activity under conditions which we can control. We must take the enzyme out of its natural environment in the liver and place it in an artificial environment, which we can keep constant. We have done this in the following way : The livers of a dog and rabbit were washed free of blood through the portal vein with ice-cold isotonic saline solution *immediately* after death. Death was caused by stunning. One portion of the blood-free liver was placed in the incubator, and from another an ice-cold saline extract was prepared. After some time a similar saline extract was made of the incubated portion. Equal quantities of the two extracts were then mixed with glycogen solutions and incubated for a certain period of time. The degree of percentile glycogenolysis for the two extracts was found to be practically the same ; there was therefore no evidence of more glycogenase in the portion of liver in which post-mortem glycogenolysis had been actively in progress—as was shown by a control analysis—than in that in which it had been prevented by cold. An objection might be raised to this experiment on the ground that the post-mortem process became established in the ice-cold extract after it was mixed with the glycogen solution and placed in the incubator. In such a case, however, the extract could scarcely develop the same amount of enzyme as that which developed in the liver itself, for the quantity of extract incubated was extremely small when compared with the mass of liver. The following is a brief survey of the results (32) :

In the experiment on the rabbit 22·5 per cent. of glycogen

disappeared in two hours in the portion of liver placed in the incubator:

Two c.c. cold liver extract + 10 c.c. glycogen + toluol showed, after five hours' incubation, 22·1 per cent. glycogenolysis.

Two c.c. extract of incubated portion of same liver, similarly examined, showed 19·1 per cent. glycogenolysis.

These observations were repeated several times, and with varying amounts of phosphate mixture, but always with the same general results—*i.e*, no distinct difference in strength between the two extracts was noticeable.

In the experiments on the dog's liver, less satisfactory results were obtained. The cold extract produced slightly less glycogenolysis than that of the incubated liver (14·5 as against 19·02 per cent.) without the addition of phosphate mixture, but the results were reversed in the presence of this (21·65 as against 18·79 per cent.).

We must conclude that the cause of post-mortem glycogenolysis is something other than a production of glycogenase. It must depend on some change which develops in the liver after death, and which facilitates the action of the glycogenase already present; or we may put it in another way by saying that the glycogenase in the liver is prevented during life from acting on the glycogen on account of some inhibiting mechanism which is removed by death.

In order to learn something regarding the nature of this controlling mechanism, we must turn our attention to the changes occurring in the liver after death that could accelerate the action of glycogenase. The first point to think of is a change in the reaction in the liver cells, for, as we have seen, small degrees of acidity accelerate the action of the diastatic enzymes, including glycogenase (p. 150). The inverting enzymes are affected by acids in the same way (33).

Although it is well known that there is a certain development of acid in the liver after death, we do not know whether this occurs at the same rate as does the glycogenolytic process, and we must know this before we can conclude that the increased glycogenolysis is dependent upon the acidity. There are many facts, however, that strongly suggest that the two processes are related. Thus, alcoholic extracts of liver* prepared from a

* There is, however, some risk in using alcohol for this purpose, for lactic acid is formed by extracting frog muscle with alcohol (Fletcher and Hopkins, 36).

portion of liver that had stood for about a day were found to be much more acid towards phenolphthalein than those prepared from the liver immediately after death, or from frozen or scalded liver (Pavy and Bywaters, 34). Similarly, the injection of sodium carbonate solution into the portal vein prevented post-mortem glycogenolysis, but this could be made to reappear by careful additions of acid. It is quite probable that post-mortem acidity may be responsible in part at least for the onset of post-mortem glycogenolysis, but there is a difficulty in accepting this conclusion without further proof because of the very rapid onset of the latter process.

Further light is thrown on this question by observing the intensity of the post-mortem process in portions of liver containing different amounts of blood or exposed in different degrees to the influence of air. It is well known that glycogenolysis proceeds much more rapidly in a portion of liver still containing blood than in one from which all the blood has been removed (35).

In such cases the glycogenase in the blood adds its effect to that present in the liver. This influence of blood is much more marked when the blood is left in the vessels of the intact viscus than when pieces of blood-free liver are mixed with blood. This fact became evident in observations on the rate of glycogenolysis in—

1. Portions of liver that were excised and placed in an incubator at body temperature.

2. Other portions placed in a pool of blood in the animal's abdomen.

3. Intact liver, in which there is no circulation of blood.

After depriving the liver of its portal blood-supply, by anastomosing the portal vein to the inferior vena cava, a large portion of liver was removed and divided in three portions ; in one of these, the original glycogen content was determined ; a second was placed in blood in the abdomen ; and a third in the incubator. After an hour the glycogen was determined in portions of the liver left *in situ*, and in each of the isolated portions. It was found that glycogenolysis had proceeded much more rapidly in the liver left *in situ* than in the isolated portions. It was also more marked in the pieces of liver placed in blood in the abdomen than in those removed to the incubator. Needless to state, the temperature of the different preparations was kept the same.

This result might be interpreted as showing that connection with the nervous system is responsible for the greater glycogenolysis in the liver left *in situ*. In an operation of the above type the only part of the nerve path to the liver which suffers damage from anæmia is the termination of the hepatic fibres in the hepatic cells. The solar plexus does not suffer, and it is possible that, for some time, the functional integrity of the connection between the nerve fibres and the liver cells might be maintained, even although there is stagnation of blood. It had to be considered whether this connection with the nervous system might account for the greater glycogenolysis occurring in the intact viscus. To put this possibility to the test, we repeated the above observations with the difference that all nerve connections to the liver were cut, but the same result as before was obtained—that is, the difference in glycogenolysis between intact and isolated liver was the same as when the nerves were intact. The nerve connections can therefore be of no importance in controlling the onset of post-mortem glycogenolysis.* The explanation for the difference is either that the blood in the intact viscus penetrates more thoroughly into the liver cells than is possible when excised portions of liver are bathed in blood, or that post-mortem acidity develops more rapidly in the intact viscus than is the case when this is cut up and exposed to air. Fletcher and Hopkins (36) have shown that the rate of development of lactic acid in frog muscle is largely dependent upon the presence or absence of oxygen ; indeed, in air, muscle often shows a diminution rather than an increase in acid, whereas this is very great under anaërobic conditions. Now, in the blood left in the bloodvessels of the liver in the above experiments, the oxygen-supply very soon became exhausted, whereas in the removed portions, being freely cut up and more or less exposed to the air in the presence of hæmoglobin, the oxygen-supply was much greater. For the same reasons, the least development of acid occurred in the incubated portions of liver, which probably accounts for the fact that in them glycogenolysis was least marked. The following Table embodies the main results upon which these conclusions are based :

* The view of Cl. Bernard that post-mortem glycogenolysis sets in on account of the removal of nerve control is shown to be wrong, because, as we have seen elsewhere, cutting the hepatic plexus does not lead to hyperglycæmia.

Post-Mortem Glycogenolysis under Various Conditions.

No. of Experiment.	Percentile Glycogenolysis in—			Difference between B. and C.	
	A.—Liver in Incubator.	B.—Liver in Blood in Abdomen.	C.—Liver left *in situ*.		
123	21·4	28·0	57·4	29·4	Hepatic nerves cut.
128	7·8	10·0	26·4	16·4	
129	—	10·1	23·4	13·3	
124	12·5	19·4	34·7	15·3	Hepatic nerves intact.
125	—	22·7	41·0	18·3	
130	—	29·8	39·4	9·6	

In the liver, isolated from the circulation as above described, the control of the nervous system, exercised through the splanchnic nerve, on the glycogenolytic functions very soon becomes abolished, for stimulation of these nerves fails to cause any change in the intensity of the process. In seeking for evidence of the existence in the splanchnic nerves of nerve fibres which could cause a change in hepatic glycogenolysis, independent of any vascular change, we examined the rate of this process before and during stimulation of the nerve in dogs in which, by the establishment of the anastomosis above mentioned, there was no hepatic circulation. Such an experiment would be analogous to that in which the existence of secretory fibres to the sweat glands is demonstrated by showing that stimulation of the sciatic nerve causes sweating from the paw in the amputated leg of a kitten.

In our earlier observations (37) two types of experiment were adopted. In the one of these the amount of glycogen which disappeared in one hour from the liver after the establishment of the anastomosis was determined in one series of dogs without any stimulation of the splanchnic nerve, and in another during such stimulation. The glycogenolysis was usually greater in the latter group. Since the hepatic arteries were not ligated in these experiments, we could not, however, be certain that the greater glycogenolysis in the "stimulation" group had not resulted from the more complete anæmia of the liver, due to vaso-constriction of the vessels ; in other words, it was possible that the supply of blood furnished by the hepatic artery had been sufficient to prevent the full development of glycogenolysis, which, however, happened when this blood-supply was curtailed by vaso-constriction. Another serious objection that can be

raised to these experiments is that the comparisons were made between the rate of glycogenolysis in the livers of different animals. By such a method a considerable experimental error is involved (see p. 116).

A second group of experiments was therefore undertaken in which, besides cutting off the portal blood-supply, that of the hepatic arteries was also abolished. In this group of experiments, a portion of liver was removed immediately after cutting off the blood-supply, and a second portion ten minutes later. The difference in glycogen between these indicated the rate of glycogenolysis in the absence of nerve stimulation. After the removal of this second portion, the splanchnic nerve was stimulated for ten minutes, and a third portion of liver then removed. The difference in the glycogen content of the second and third portions of livers indicated the degree of glycogenolysis occurring during nerve stimulation. Although every precaution was taken, by the employment of duplicate analyses, etc., to avoid technical errors, the results were very irregular. The average rate of percentile glycogenolysis during the first ten minutes, in the seven experiments performed, was 22; during the second ten minutes, in two experiments in which the splanchnic nerve was not stimulated, it was 12·4; and in three, in which such stimulation was performed, it was 28·2. These average results would seem to indicate that the nerve stimulation had caused an acceleration of the glycogenolysis, but our subsequent observations on the course of post-mortem glycogenolysis, revealing great irregularities in the process, have compelled us to conclude that the evidence offered by those experiments is unreliable (see p. 116). I have thought it well, however, to say something about them so that others interested in the same problems may have the benefit of our experience.

We have finally succeeded in showing very clearly that no change is produced in the rate of glycogenolysis by stimulation of the splanchnic nerve in a liver deprived of its blood-supply. We have effected this by experiments in which the difference in percentile glycogenolysis occurring in liver left *in situ* was compared with that in a portion of the same liver placed in blood in the abdomen. The average difference between the two was found to be practically the same when the splanchnic nerve was stimulated as without such stimulation (19).

Any one of three conclusions is possible from these results:

(1) That there are no fibres in the splanchnic nerves which are capable of directly influencing the glycogenolytic process in the liver, the control exercised by this nerve being an indirect one, through its vasomotor fibres. (2) That the functional integrity of the hepatic nerve endings is abolished by deprivation of the blood-supply. (3) That the intensity of the glycogenolysis set up in the liver by deprivation of its blood-supply is as great as it can be under any circumstances, so that nervous influences can produce no further changes in it.

Before leaving this somewhat unsatisfactory part of our researches, I should like to point out that the influence which we have recently found the adrenal glands to exercise over the functional integrity of the hepatic nerve terminations in the liver cells would in itself render it certain that in the above experiments, as also in those with perfused livers, it is useless to expect that stimulation of the splanchnic nerves will have any influence over the glycogenolytic process, even supposing that this is not already interrupted by interference with the blood-supply.

It has for long been a much-debated question whether the cause of increased glycogenolysis during life is the same as that of the post-mortem process. Pavy constantly maintained that the post-mortem process is due to the development of conditions which do not exist in the living animal. He believed that in the living animal the glycogen does not ordinarily become retransformed into dextrose, but changes into other substances. If this were true—which, of course, it cannot be—then the study of post-mortem glycogenolysis would be of little interest.

The first step towards showing whether the ante-mortem and post-mortem forms of glycogenolysis are due to the same causes must consist in a study of some conditions which are intermediate between the two. Such an intermediate condition is that produced by disturbances in the circulation of blood through the liver, and in studying the effect of this on the glycogenolytic process we do not require to measure the amount of glycogen in the liver from time to time, but can employ, as a criterion of the rate of glycogenolysis, the changes which occur in the amount of sugar in the blood. This is most accurate when the blood of the vena cava is used. Three types of vascular disturbance in the liver have been investigated in this way—namely : (1) Ligation of the hepatic arteries ; (2) temporary clamping of the portal

vein; and (3) splanchnic vaso-constriction, brought about by stimulation of the splanchnic nerves after complete division of the hepatic plexus. In the first two cases the sugar of the arterial blood was examined; in the last case, that of the vena cava as well.

No change was produced in the amount of sugar in the arterial blood, even in over two hours after complete ligation of the hepatic arteries (38). Of course, the blood supplied to the liver through these vessels is very small when compared with that which is supplied through the portal vein, but it is rich in oxygen, and it was considered of importance to see whether a vascular disturbance, even so small as this, might not be sufficient to excite glycogenolysis.

After temporary clamping of the portal vein, very different results were obtained; by this procedure, glycogenolysis became very readily established. As we have elsewhere remarked, it is by this method that the most accurate information regarding the exact moment of onset of post-mortem glycogenolysis is likely to be obtained. So far as our results at present go, they show that clamping of the vein for one minute, with intervals of two minutes between the periods of clamping, is sufficient to excite the process. Thus, in a specially fed dog the percentage-reducing power in the arterial blood rose from 0·211 to 0·352 in forty-five minutes, during which the portal vein was intermittently clamped, as above described. Three similar experiments yielded concordant results. In one experiment the vein was only partially clamped, the clamp being, however, permanently applied, but the increase in blood-sugar was not so marked as in the other cases.

Evidently, therefore, hepatic glycogenolysis is very readily induced by disturbances in the portal circulation. This conclusion may not at first sight seem to be corroborated by the observations that have been made on animals with an Eck fistula. Such animals can be kept alive for a considerable time, even although the portal blood fails to pass through the liver; but during no stage do they exhibit glycosuria. The reason for this is that, although extensive glycogenolysis is going on in the liver, this sugar cannot joint the general circulation, because there is no blood passing through the vessels to carry it away. It becomes gradually transferred to the general circulation by means of the anastomotic vessels which afterwards develop, or by

diffusion into the blood of the hepatic veins. Likewise no glycosuria is commonly observed in patients suffering from thrombosis of the portal vein, or in other clinical conditions of portal obstruction. In such cases there is no hepatic circulation to wash the sugar out of the liver.

More moderate degrees of disturbance in the portal circulation do not appear to excite glycogenolysis ; thus, stimulation of the great splanchnic nerve, after cutting all the fibres of the hepatic plexus, must cause considerable changes in the volume flow through the portal vein (as a result of the constriction of the splanchnic bloodvessels). But, as we have seen elsewhere, it causes no change in the amount of reducing substance, either in the arterial blood or in that of the vena cava (see p. 66).

When, during the course of an experiment, the arterial blood-pressure becomes considerably lowered, we have always found that the process of glycogenolysis in the liver becomes excited, as evidenced by the occurrence of hyperglycæmia. The cause of this is clear in the light of the above results. We have made no systematic investigation of the exact level to which the blood-pressure must fall before this effect on the glycogenolytic process makes itself evident, but we have learned to disregard, on this account, all results obtained with arterial pressures below 60 millimetres of mercury. By an examination of the amount of reducing substance in the urine, it appeared, in our earlier researches, as if a considerable fall in arterial blood-pressure had the opposite effect to that described above—that is to say, that it caused a decrease in the glycosuria (39).

We now know that this is because of the depression in the excretion of urine (see p. 46). It seems to be the case that, under these conditions, the urine excreted contains a smaller percentage of sugar, even although that present in the blood is steadily mounting.

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CHAPTER VII

THE PROBABLE CAUSE OF HYPERGLYCÆMIA IN VARIOUS FORMS OF EXPERIMENTAL DIABETES

Since the variations which occur in the process mobilization of sugar in the liver are not due to changes in the amount of the enzyme (glycogenase), we must conclude that the environment changes in which the glycogenase acts. There are several conditions of the environment which can be regarded as responsible for the change, such as concern either the condition of the glycogen or the activity of the glycogenase. In connection with the former possibility, it must be borne in mind that glycogen is not in solution in the liver cell, but is deposited in masses within its protoplasm. The glycogen in these masses is supposed by some histologists to exist in a form of combination with a sustentacular substance (Arnold, 1), and it is readily conceivable that, while thus fixed, it cannot be attacked by glycogenase, but immediately becomes so when it is set free. According to this view, the influence of the nervous system, as well as that of various other conditions which can effect the glycogenolytic process, would be centred on the degree of union between the glycogen and the sustentacular substance. This hypothesis can be supported by some interesting facts. Thus, during a certain stage of phosphorus-poisoning, the injection of dextrose does not cause any glycogen to be deposited in the liver, but produces a hyperglycæmia which persists for a much longer time than is the case in a normal animal. Meanwhile, we shall devote our attention to the other possibility—namely, that it is because of changes in the environment of the glycogenase in the liver cell that its variable activities are due.

It will be remembered that the rate of glycogenolysis in incubated mixtures of glycogenase and glycogen or starch is most markedly affected by the addition of alkalies or acids. The

effect of neutral salts, especially of sodium chloride, does not appear to be so marked as in the case of saliva. The statement of Bang that such substances as lecithin have an influence has not been confirmed ; on the contrary, it has been found that fresh extracts of liver have the same glycogenolytic power as extracts that have been freed of such substances by treatment with toluol (2A). Nor is the stimulating effect of adrenalin on hepatic glycogenolysis due to any direct action of this substance on glycogenase, for we have found its addition to a fermentation mixture has no influence on the glycogenolysis. Even fresh saline extracts of the adrenal glands are without effect. As pointed out before, the adrenalin acts on the nerve terminations.

It is highly probable, however, that alterations might occur in the environmental conditions which it is impossible for us to duplicate in ferment mixtures, and it becomes of importance, therefore, to see whether we can bring about such changes in the liver itself by injecting various substances into the blood of the portal vein.

The extreme susceptibility of glycogenase to acid or alkaline reaction (see p. 150) suggests the importance of studying the effect on the sugar output of the intact liver produced by changes in the reaction of the circulating blood. This can be done by injecting weak acids and alkalies into the blood of the portal vein. The author has performed a few such experiments, using for this purpose eighth molecular solutions of lactic acid, and injecting slowly such quantities as would just stimulate the respiratory centre. Examination of the arterial blood did not, however, reveal any increase in reducing substance.

These preliminary experiments are not to be considered as at all conclusive, however, because quite probably an amount of acid was injected that was far beyond the optimum which, as we have already seen, is reached with quite a low acid concentration (see p. 150). However, there is no mention made of the occurrence of hyperglycæmia or glycosuria in the writings of those who have studied the general problem of acid intoxications, and neither condition is known to develop in mountain sickness which is associated with a diminished alkalinity of the blood.

It is possible that valuable information regarding the influence of environment on the activity of glycogenase could be obtained by a systematic investigation of the sugar content of the blood

of the vena cava following the injection of other substances than acids and alkalies into the blood of the portal vein. Since such studies so far have not been made, the only means left to us by which further light can be thrown on the effect of altered environment on the activity of glycogenase is to observe the behaviour of certain forms of experimental hyperglycæmia, which, undoubtedly dependent upon an increased glycogenolysis, are yet unaccompanied by evidence of change in the amount of glycogenase in the liver. To establish this latter point, it is not necessary that we subject every one of the many varieties of experimental hyperglycæmia to a separate investigation; a few types serve for all the others. Besides those produced by stimulation of the splanchnic nerve and by post-mortem change, we have examined those due to asphyxia and ether, and Starkenstein has examined that due to piqûre (2A); and since in none of them has any change in the amount of glycogenase been found, we have provisionally concluded that there is no form of hyperglycogenolysis that is dependent upon an increased production of this enzyme in the liver.

The form of hyperglycæmia and glycosuria which we shall first of all consider in this connection is that produced by *asphyxia*, in which, besides anoxyhæmia, there is a marked change in the reaction of the blood due to the accumulation of carbon dioxide, and of incompletely oxidized acid substances (2).

The points to which we must pay attention are these:

1. The intensity and duration of the hyperglycæmia and glycosuria. In this connection we must see whether interference with respiration may not be the real cause of the hyperglycæmia, which is produced by certain drugs and by conditions that excite the nerve centres.

2. The evidence that the hyperglycæmia of asphyxia is due to hepatic hyperglycogenolysis.

3. Whether the asphyxial condition exerts its influence directly on the liver, or indirectly through its effect on the nerve centres.

4. Whether it is oxygen deficiency or carbon dioxide accumulation that is responsible for the hyperglycogenolysis.

An account of the intensity and duration of the hyperglycæmia and glycosuria need not occupy much of our time. Both are more or less proportional to the intensity of the asphyxia. To produce the asphyxia in my own experiments (3), the respira-

tion-tube connected with the trachea in etherized dogs was occasionally obstructed until the several stages of asphyxia had become well developed, as judged from the curve of arterial blood-pressure. In a typical experiment hyperglycæmia became marked in thirty minutes—viz., 0·296 per cent.—and increased in degree so long as the asphyxial periods were kept up. At least this was the case up to two and one-half hours after the first asphyxial period, when the percentage of reducing substance stood at 0·424. Corresponding degrees of diuresis and glycosuria accompanied the hyperglycæmia, just as was found to be the case when the splanchnic nerve was stimulated (see p. 46) In order to ascertain the after-effect of the asphyxia, the asphyxial periods were discontinued in one experiment after one and one-half hours, and the animal kept under observation for a further period of two and one-half hours. Frequent examination of the urine* showed that for thirty minutes after the discontinuance of asphyxia, the percentage of reducing substance went on rising, after which it gradually fell, until, in two and one-half hours, it stood at about one-half its previous level. The rate of urine formation varied in the same general way as the glycosuria.

Prior to my own experiments, very similar ones had been performed by Underhill (4). This author produced dyspnœa in some of his experiments by clamping the tracheal tube, and in others by injecting a paraffin mixture into one carotid and ligating the corresponding artery on the opposite side. By this latter procedure the respiratory movements ceased, because of anæmia of the respiratory centre, and the animals had to be kept alive by artificial respiration. The hyperglycæmia became very marked (0·38 to 0·42 per cent.) in the second group of experiments.

The main importance of these observations is in connection with the possibility that many forms of glycosuria and hyperglycæmia, which are ordinarily supposed to be due to entirely different causes, may really be asphyxial in nature (see p. 78). Of course, in the two experiments mentioned above, the asphyxia was quite marked ; but so was the hyperglycæmia, and it is not unreasonable to assume that lesser degrees of asphyxia would be accompanied by lesser degrees of hyperglycæmia and glycosuria.

* The blood was not examined because to have done so would have complicated the result on account of the hæmorrhage.

The forms of experimental hyperglycæmia which may depend upon the development of an asphyxial condition include experiments on the upper portion of the spinal cord and stimulation of sensory nerves. In both types of experiment considerable disturbance of the respiratory movements is inevitable, this being especially the case when the cervical portion of the spinal cord is stimulated. Under such conditions, and without any precautions against asphyxia, the average percentage of reducing substance in the arterial blood of five dogs rose from normal to 0·256 in about forty-five minutes after the first application of the stimulus. On the other hand, in six dogs, in which the cervical portion of the spinal cord was stimulated in exactly the same way as above, but the development of an asphyxial condition was guarded against by artificial respiration and the intratracheal insufflation of oxygen, no change in the percentage of sugar of the arterial blood was observed in an average time of twenty-six minutes after the application of the stimulus.

By stimulation of the central end of the vagus nerve very similar results were obtained. When no precautions against the development of an asphyxial state were taken, hyperglycæmia became established in about an hour after the first application of the stimulus ; thus, in seven dogs the reducing power rose to an average of 0·222 ; but in eight other dogs, in whom the development of the asphyxial condition was guarded against, there was only a slight increase in the blood-sugar ; thus, in an average time of ninety-six minutes after the first application of stimulation, the average percentage of sugar was 0·170. Of course, as already pointed out, these observations do not necessarily disprove the existence in the vagus nerves of afferent fibres which influence the glycogenic centre ; they indicate, however, that their presence has not as yet been satisfactorily demonstrated.

Many drugs that produce hyperglycæmia do so as a result of interference with the respiratory function. Thus coniin, nicotin, piperidin, and pyridin, besides causing hyperglycæmia, exert an influence on the respiratory centre, which may be so marked as to produce respiratory failure, and, in the case of piperidin (painted on the pancreas), Underhill (5) succeeded in showing that no hyperglycæmia became developed when the animals were made to respire from a mask, through which abundance of oxygen was administered.

Other observers (for references, see 3) have ascribed to asphyxia the hyperglycæmia which curare produces ; for this drug, in paralyzing the so-called motor end-plates of muscles, causes paralysis of the respiratory movements. In my own experiments with curare the hyperglycæmia was extremely marked (without there being any glycosuria or diuresis), when the artificial respiration was no more than just sufficient to keep the animal alive ; but was much less pronounced when, by oxygen insufflations as well as artificial respiratory movements, the tendency to asphyxia was guarded against. For example, in one dog treated in the former manner, the percentage-reducing power of the arterial blood rose from 0·158 to 0·613 in an hour, whereas in two other animals in which abundance of oxygen was given, it rose only to 0·232 in ninety minutes in one case, and 0·262 in sixty minutes in the other. The artificial respiration had greatly diminished the development of hyperglycæmia, although it had not entirely prevented it. The glycosuria following respiration in an atmosphere containing carbon monoxide is probably also due to asphyxia (Zuntz).

Asphyxial hyperglycæmia is dependent upon the liver, for when this is removed from the circulation, as by anastomosis of the portal vein with the vena cava, mechanical asphyxia is without effect on the sugar in the blood. Out of a total of seven experiments in which this operation was performed, and in which frequent attacks of asphyxia were induced by clamping the respiration-tube, the percentage of sugar in the arterial blood did not rise above 0·220 in six of them—the average being 0·176—even in an hour after the first application of the clamp. In one experiment, however, a distinct hyperglycæmia did become established, but it is uncertain whether in this case the hepatic artery had been ligated. The same was observed for the asphyxia produced by curare ; in three dogs with Eck fistulæ, and to whom the drug was given until the muscles were paralyzed, not even the slightest increase in the amount of reducing substance in the blood was observed.

It is of interest in this connection to note that removal of the liver from the circulation also abolishes the glycosuria which is produced in the normal animal by piqûre, hæmorrhage, and pancreatectomy. When it is removed from a normal animal, however, the blood-sugar does not, as was

previously thought to be the case, entirely disappear (for references, *vide* 3).

The most natural conclusion to draw from these results is, of course, that asphyxia stimulates the glycogenolytic process in the liver. It cannot apparently stimulate a similar process in the muscles, where there are also large stores of glycogen. This fact is one of several indicating that there is some physiological difference between the glycogen deposited in these two places.

Several writers have concluded that the mechanism which is responsible for asphyxial hyperglycæmia is a depressed utiliza tion of dextrose in the blood or tissues (Lepine and Boulud, 6), which results either because a poisonous substance, or "leucomaine," becomes developed in the blood, on the glycolytic power of which it has a depressing influence; or because the oxygen deficiency depresses the activity of the tissue oxidases (Underhill, 5). In either of these cases, however, it is difficult to see why removal of the liver from the circulation should prevent the hyperglycæmia, unless it be that the glycogen stores in the muscles are entirely unavailable for the purpose of supplying dextrose to the blood.

We may therefore conclude that the asphyxial condition leads to hyperglycæmia, because it excites the glycogenolytic process in the liver. We must inquire now as to how it does this. The possibilities to be considered are (1) by direct action of the asphyxial blood on the liver; or (2), indirectly, through its action on the glycogenic nerve centre.

The method adopted for investigating these questions has consisted in cutting the fibres of the hepatic plexus in the manner described elsewhere. Unfortunately, however, the outer coat of the portal vein was not seared so as to insure complete severance of all the fibres (see p. 66).

Out of a total of eight experiments on dogs, in which, after such division of the hepatic nerves, mechanical asphyxia was induced, a marked hyperglycæmia became developed in the arterial blood within an hour in two, a slight degree of this in four, and no change whatsoever in two.

The results of similar experiments in which curare was injected were much more definite. There were four such experiments, and in all of them it was found that undoubted hyperglycæmia occurred after the division of the nerves.

To explain the absence or slight degree of the hyperglycæmia following division of the nerves in six of the cases of mechanical asphyxia, and its persistence in the other cases, including those curarized, we may assume that asphyxia excites hyperglycogenolysis of slight degree through the nervous system alone, and that the more intense degrees (such as are produced by curare) result from a direct action on the glycogenolytic mechanism in the liver. The general stimulating effect of asphyxial blood on the so-called "vital centres" evidently involves that centre which controls the glycogenic mechanism; hence it is difficult to decide whether there may be *afferent*, in addition to direct, stimulation of the glycogenic centre. The observation that intense degrees of asphyxia can still cause hyperglycæmia after section of the hepatic nerves is of very great interest, illustrating as it does the influence which changes in the environmental condition may have on the activity of glycogenase. There are several properties of the asphyxial blood which might be held responsible for this action, such as deficiency in oxygen, excess of acids including carbon dioxide, and the presence of incompletely oxidized metabolic products. It is a comparatively easy matter to show that an excess of carbon dioxide in the blood excites the glycogenolytic process, and that the mere absence of oxygen does not have this effect. In order to show this, the liver of a dog or rabbit was excised immediately after death, cooled to near freezing-point, and finely minced. Equal amounts of the mince were then placed in three pairs of small Erlenmeyer flasks, and mixed with equal quantities of defibrinated blood. The glycogen was immediately determined in one pair, and the others were submersed in a water-bath at body temperature, moist air or oxygen being bubbled through the contents of one pair, and moist carbon dioxide, hydrogen, or coal gas, through that of the other. After a certain period of time—which varied from one to four hours in the different experiments—the flasks were simultaneously removed from the water-bath, and their contents mixed with equal volumes of 60 per cent. caustic potash, and the glycogen estimated in the usual manner.

Leaving out all details, and expressing the amount of glycogen which disappeared from the various flasks as a percentage of the amount present in the control flasks, the following results were obtained :

Percentile Glycogenolysis in Oxygen.	Percentile Glycogenolysis in Atmospheres Indicated.	Duration of Incubation.
46·50	CO_2 58·80	4 hours
23·02	CO_2 32·57	2 ,,
56·12	H 59·21	3 ,,
37·30	H 36·16	3 ,,
32·70	Sealed flask 31·66	3 ,,
28·00	Coal gas 33·00	50 minutes

It is clear that glycogenolysis proceeds at the same rate in the presence of oxygen or hydrogen, but quicker in the presence of carbon dioxide. This result is exactly what we should expect from our knowledge of the influence of small degrees of acidity on the glycogenolytic process (see p. 150), and it is in strict conformity with the observations of Schierbeck (7), who studied the influence of carbon dioxide on the glycogenolytic action of saliva. We cannot, of course, conclude from the results that the direct action of asphyxial blood in producing hyperglycogenolysis is *solely* dependent upon the presence of an excess of carbon dioxide in the blood, for, as a result of deficient oxidation, other acid substances are produced in this condition, and these will reinforce the action of the carbon dioxide. It is probably correct to state that an increase in the real acidity of the blood is the cause of the hyperglycogenolysis of asphyxia; or, to put it in the language of physical chemistry, it is due to an increase in the H·-ion concentration.

The above results clearly show that such acid substances do not become developed in sufficient quantity to stimulate the glycogenolytic process in defibrinated blood kept in an atmosphere of hydrogen; those which develop in conditions of deficient oxidation in the body must be extremely labile, for it is quite impossible to show that blood removed from an asphyxiated animal differs in its influence on the rate of glycogenolysis conducted outside the body from that of arterial blood; and this is true even when every precaution is taken to prevent contact of the asphyxial blood with oxygen. Thus, blood was collected from an asphyxiated dog into a flask filled with hydrogen, in which it was defibrinated. This blood was then transferred through glass tubes filled with hydrogen into other flasks containing the minced liver in the presence of hydrogen. The mixture of blood and liver was incubated for a certain time, hydrogen gas being

meanwhile bubbled through it. In other flasks, similar quantities of arterial blood from the same dog and of the same minced liver were incubated with oxygen bubbling through the mixture. In two experiments the percentile glycogenolysis, after three hours' incubation in the arterial blood, was 45·5 and 48 per cent., and in the asphyxial (hydrogen) blood, 42·7 and 48 respectively. Of course it must be remembered that the conditions in these experiments are very different from those that obtain in the intact animal. In the one case, only a small unchanging amount of blood comes in contact with the liver; in the other, large volumes are constantly circulating through it. The traces of acid substances present may be capable of making their influence felt only when large volumes of blood are brought in contact with the liver.

In mild degrees of asphyxia the various phenomena associated with it are dependent solely upon an increased tension of carbon dioxide in the blood, and therefore on a slight rise in the H··ion concentration. The nerve centres become excited by this—the cerebral first, and the bulbar later. The spread of the stimulus to these involves that which is in control of the glycogenic function. Later, the effect spreads to the spinal centres, and, by producing muscular convulsions, leads to the appearance of lactic acid in the blood; so that the acidity of this reaches such a degree that the glycogenolytic function in the liver becomes directly stimulated.

The increased glycogenolysis that is produced by muscular exercise is very likely associated with the accompanying rise in the tension of carbon dioxide in the blood, acting through the glycogenic centre. When the exercise is so severe that oxidation does not keep pace with it, and lactic acid therefore appears in the blood, glycogenolysis probably becomes still more stimulated by direct action on the liver, and it may be on this account that, after exhausting muscular exercise, the glycosuria in diabetic patients undergoes an increase instead of a decrease, as is the case when the exercise is moderate in degree.

It has been a controversial question as to whether it is the deficiency of oxygen or the excess of carbon dioxide in the blood that is immediately responsible for the glycosuria produced by asphyxia. Araki (2) believed that it is because of oxygen deficiency, and Edie (8) that it is because of accumulation of carbon

dioxide. Both views are probably correct, for a deficiency of oxygen in the respired air will lead to the production of acids in the organism, and these, if they do not themselves accumulate in sufficient amount to have a stimulating effect on glycogenolysis, will at least reinforce the action of the carbon dioxide.

Looked at from this point of view, it becomes an easy matter to explain why intratracheal insufflation of oxygen should prevent or retard the development of asphxyial hyperglycæmia. The high tension of oxygen in the alveolar air will, besides keeping the hæmoglobin saturated, raise the amount of oxygen present in simple solution in the plasma, thus insuring that no unoxidized acid substances make their appearance ; and, moreover, the constant stream of oxygen in the bronchi, coupled with the artificial respiratory movements, will insure the thorough removal of carbon dioxide from the alveoli.

It is said that asphyxia does not cause glycosuria in rabbits after removal of the adrenal glands. This would indicate that the stimulus of asphyxial blood can act on the glycogenic function in the liver only when the functional integrity of the nerve path is maintained by a certain concentration of adrenalin in the blood. There may also be a hypersecretion of adrenalin during asphyxia, for histological changes occur in the glands in this condition (Starkenstein, 8A). Our inability to prevent curare from causing hyperglycæmia, even by administering large quantities of oxygen and maintaining energetic artificial respiratory movements, may be due to the development in the blood in this condition of (acid) substances which are unoxidizable, and which stimulate the glycogenolytic process, or it may be that the asphyxia produced by this drug is so intense that it cannot be overcome by artificial means.

Another condition which leads to the development of glycosuria, and which is probably more or less associated with changes in the environmental condition of the liver cell, is the presence of an excess of sodium salts in the blood. The sodium salt may be injected in solution either into an artery or into a vein, the most striking results being obtained when rabbits are used (9). The addition of a small amount of calcium chloride to the sodium chloride solution prevents the glycosuria. There seems, however, to be a fundamental difference between the condition produced by intravenous and intra-arterial injection, and to explain

this Fischer concluded that the sodium salt produces two effects that are more or less independent of one another—the one, a stimulation of the glycogenic centre, and the other a diuretic influence on the kidneys (10). His only experimental evidence for this conclusion is that glycosuria occurs much more quickly when the injections are made through the central end of the axillary artery than when they are made intravenously. By the former path the salt will be carried by the vertebral arteries directly to the medulla ; by the latter, the sodium salt will not reach the medulla until it has been more or less diluted by blood, but it will more quickly act on the kidneys. These conclusions were, however, drawn without any estimations being made of the amount of reducing substance in the blood. Conducting such estimations, Underhill and Closson (11) made the very interesting discovery that intravenous injection of solutions of sodium chloride, besides causing glycosuria and diuresis, lead to the development of *hypo*glycæmia, which cannot be accounted for by the dilution of the blood, because this is more or less prevented by the accompanying diuresis.

These authors concluded that the glycosuria, under such conditions, is probably due to an action of the sodium ion on the kidney, rendering it more permeable towards the sugar in the blood, and thus accounting for the hypoglycæmia. Sodium chloride is well known to have a stimulating effect on the kidney activity, and calcium to be capable of neutralizing this effect ; and in agreement with this, Underhill and Closson found that, after glycosuria had become established by injecting sodium chloride, the subsequent injection of a mixture of 975 c.c. $\frac{M}{6}$ NaCl and 25 c.c. $\frac{M}{\frac{3}{8}}$ $CaCl_2$ causes the glycosuria gradually to disappear (the diuresis becoming less marked), and the blood to reacquire its normal percentage of reducing substance.

A very different mechanism is described by these authors as responsible for the glycosuria which occurs when the salt injections are made into the arterial system in such a way that the salt is carried more or less directly to the cerebral circulation. Marked interference with the respiratory movements results from such injections, and hyperglycæmia is developed. It seems probable, therefore, that we may have to deal with an asphyxial hyperglycogenolysis. But there are innumerable details that

remain to be worked out in connection with this interesting form of glycosuria. It is stated that injections of sodium chloride solutions fail to cause glycosuria after division of the splanchnic nerves. This would seem to indicate that the action of the sodium chloride must be on the glycogenic nerve centres, and not directly on the liver, but the researches should be repeated so as to include estimations on the blood-sugar, for the disappearance of glycosuria may be dependent on the low blood-pressure following section of the splanchnic nerves (see p. 46). It is possible that the action may be centred directly on the liver—in other words, that excess of sodium (chloride) has a stimulating action on the glycogenolytic process. If this is the case, we should expect to find that injections of sodium chloride solution in the portal vein will cause a higher percentage of reducing substance to appear in the blood of the vena cava opposite the liver than is the case when equal quantities of a saline solution containing calcium are injected.

Anæsthesia is sometimes followed by glycosuria, but the changes in the amount of sugar in the blood are very slight. The form of anæsthesia which has been most thoroughly investigated in this regard is that induced by ether. In man, such anæsthesia, maintained even for two and a half hours, does not cause dextrose to appear in the urine (12); but in dogs it sometimes causes a gradual increase in the reducing substance in the blood, which, however, is very slight, and can usually be discounted in experiments performed on these animals. The urine of etherized dogs not infrequently acquires abnormal reducing powers, without there being any detectable hyperglycæmia. It was pointed out by Seelig (13) that it is only flesh-fed dogs that exhibit the glycosuria after ether; in those fed on carbohydrates it does not occur. In the former group of animals the glycosuria became established in from ten minutes to two hours after starting the administration of the ether, and it very quickly disappeared after this was discontinued. Seelig was able, further, to demonstrate that the mere act of tying the animal to the dog-board was not responsible for the glycosuria, nor could he observe any parallelism between the rectal temperature and the glycosuria (*cf.* 14). Examination of the blood revealed only a slight degree of hyperglycæmia. The passage of a stream of oxygen into the blood of the vein (at the rate of 20 to 50

centimetres per kilogramme per hour) prevented the glycosuria, but was incapable of causing it to disappear when once it had become established.

My own experience would go to confirm Seelig's findings, and some of the results, along with others by Underhill, are incorporated in the following table :

Per Cent. Reducing Substance at Beginning of Observation.	Per Cent. Reducing Substance after being under Ether for Periods indicated.	Source of Blood.	Remarks and References.
0·148	0·196 (30 minutes)	Arterial	Spinal cord exposed.
0·153	0·180	,,	Mixed diet.
0·179	0·181	,,	Mixed diet.
0·166	0·146 (70 minutes)	Vena cava inferior	Dog-fed sugar.
0·165	0·124 (80 ,,)	,, ,,	,, ,,
0·194	0·213 (30 ,,)	,, ,,	,, ,,
0·151	0·121 (20 ,,)	,, ,,	,, ,,
0·160	0·180 (3 hours)	Arterial	Underhill (*Jour. Biol.*, chap. i., p. 1. Meat Diet.
0·160	0·180 (3 ,,)	,,	Do. Do.

In the earlier experiments the dogs employed were not especially fed with carbohydrate, but mainly with flesh, and in them there was usually a moderate but steady increase in reducing substance in the blood, and the urine usually acquired reducing properties. All of the later observations were made on animals that were given, on the evening preceding the experiment, a considerable amount of cane-sugar by stomach-tube. In these, as the table shows, it almost always happened that the reducing substance in the blood (of the inferior vena cava) became less.

Hawk, while confirming the above results, has also made the interesting observation that the purity of the ether is not related to the incidence of the glycosuria. This author has also collected many interesting facts regarding other conditions that affect post-anæsthetic glycosuria (15).

The changes in the amount of reducing substance in the blood would lead us to expect corresponding changes in the process of sugar production in the liver. It will be remembered that the most valuable criterion of the activity of the glycogenolytic process is furnished by a determination of the velocity constant, the necessary data for the calculation of this value being furnished by estimating the glycogen in portions of liver

removed at different periods of time. In order to determine the magnitude of the constant during ether anæsthesia, we must, of course, have some standard with which to compare it, and the only one available is that of post-mortem glycogenolysis; for we cannot obviously use for this purpose the rate of glycogenolysis occurring in an unanæsthetized animal.

The experimental procedure which we have used has consisted in removing, at regular intervals of time, portions from the liver of dogs fed previously with bread, meat and sugar, and kept under light ether anæsthesia. After about one and a half hours the animals were killed, but left on the warmed operating-table, portions of liver being removed for analysis at intervals of fifteen minutes. The removed portions of liver were freed as far as possible of blood, weighed, and the amount of glycogen determined. The results of three such experiments are given in the following table, in which the percentage amounts of glycogen that disappeared in a given time interval are compared before and after death, and the velocity constants calculated:

Number of Experiment. Condition of Animal.	Percentage Amount of Glycogen disappeared in Equal Time Periods.	Velocity Constants. ×10 -4.
I.		
Ether anæsthesia ..	1·38	15·4 14·1 17·7
Death by hæmorrhage ..	1·21	25·0 23·6 — 18·7
II.		
Ether anæsthesia ..	1·25	7·7 — — 10·5 11·2
Death by chloroform ..	0·99	11·5 10·0 10·6
III.		
Ether anæsthesia ..	1·23	— — 31·7 24·3 27·4
Death by hæmorrhage ..	1·60	42·7 47·7 44·8 47·0

* T = fifteen minutes. The dashes indicate that no constant was calculated for that period.

These values are approximate, because of the very variable speed at which the glycogenolysis probably occurred in different lobes (p. 117), but they are constant enough to show us that there exists, during ether anæsthesia, a glycogenolysis that is not so very much less in degree than that which sets in after death.

How, then, are we to harmonize these apparently contradictory observations—viz., that the glycogen rapidly disappears without there being any increase (indeed, in the case of sugar-fed dogs, a decrease) in the percentage of reducing substance in the blood ? We can do so only by assuming that the glycogen may leave the liver, and be discharged into the blood in some other form than sugar—possibly as some dextrin, which is either precipitated along with the proteins or is unable to reduce cupric salts. This possibility is not unsupported by fact. It has been observed, for example, that there may be more actual sugar in the blood of the carotid artery than in that of the right ventricle, and that the increase in reducing power which is produced by boiling the blood-extract with an acid is more marked in the venous than in the arterial blood (see p. 45). Lepine interpreted these results as indicating that there must exist in the blood as it issues from the liver some form of compound (glucoside ?) in which the sugar is locked away, to become liberated, however, before the blood has traversed the lesser circulation. It is possible that it is as some dextrin that the glycogen in these conditions leaves the liver, and that this becomes converted to sugar by the blood-diastase. On this account it is, perhaps, unfortunate that it should have been vena cava blood instead of arterial blood that was used for analysis in the dogs that were fed on carbohydrates in our experiments.

Very suggestive results of the same nature have also been brought to light with regard to the action of phosphorus on the glycogenic process. At an early stage in phosphorus-poisoning Frank and Isaac (16) have found that there occurs a decrease in the reducing substance in the blood, accompanied by a rapid disappearance of glycogen from the liver and an accumulation of fat.

It is believed that the initial effect of phosphorus on the liver cell is to stimulate its metabolic processes, one result of this being that the glycogen, after passing through a dextrose stage, becomes converted into lactic acid.

In the later stages of phosphorus-poisoning other derangements

in the glycogenic function of the liver appear—first of all an inability of the liver cell to fix the glycogen (Neubauer, 17), so that, when dextrose is injected in the circulation, the hyperglycæmia which is thus caused persists for much longer than in normal animals, and later a disappearance of the power to synthesize carbohydrate out of amino-acids, etc. (glyconeogenesis).

There are possibly many other conditions of poisoning by drugs in which disappearance of glycogen is unassociated with the development of hyperglycæmia, and it would undoubtedly be of great value to know much more than we do at present regarding the relationship between the two. From the above observations regarding this relationship in ether anæsthesia and in phosphorus-poisoning it can at least be concluded that *hyperglycogenolysis is not necessarily accompanied by hyperglycæmia.*

To take up in detail all of the other conditions which may lead to disturbances in carbohydrate metabolism would be of little profit, but to complete this chapter it may be well to say a few words concerning the effect of hæmorrhage and of muscular work on the control of sugar in the blood.

The effect of *hæmorrhage* has been made the subject of special investigation within recent years by Rose (18), Schenck, Andersen, Nishi, and Rona and Takahashi. Many of the experiments have been performed on rabbits, in which, being small animals, the effects of the removal of even small amounts of blood become very marked. Some of the most interesting results concerning the behaviour of the blood-sugar are as follows: Schenck (19) found that, after the removal of 25 c.c. of blood (which in an ordinary rabbit constitutes from one-fourth to one-third of all the blood), an appreciable hyperglycæmia became developed in about fifteen minutes, but subsided after a couple of hours. In starved animals, or in those in which the liver had been removed from the circulation, hæmorrhage did not have this effect. Andersen (20), also using rabbits, obtained more striking results, a distinct hyperglycæmia being evident in five minutes after the removal of about one-tenth of the total blood-volume. (The percentile increase in blood-sugar was found in some cases to amount to 64·5 in five minutes, and 91·7 after thirty minutes.) By fermentation with yeast and titration of the fermented solution with Bang's solution, it was thought that some of this sugar

was other than dextrose. We have, however, seen how such a result is probably due to experimental fallacy.

Nishi (21) made the very important observation that hæmorrhagic hyperglycæmia still occurs after section of the splanchnic nerves on both sides, and after double adrenalectomy. The former fact would indicate that the glycogenolytic process in the liver must become directly stimulated by the blood-depletion, possibly by the same general mechanism as that which is responsible for the hyperglycogenolysis produced by extreme asphyxia and by clamping of the portal vein. The latter observation is of interest as illustrating one of the few conditions capable of inducing hyperglycæmia after adrenalectomy (see p. 73).

Another condition which can also do this is lowered blood-pressure. The observations serve as important evidence for the view, which we have already expressed, that there are at least two essentially different types of conditions that can stimulate hepatic hyperglycogenolysis—one typified by the control of the nervous system, the other by disturbances in the blood-circulation of the liver. It is probably the case that the fine adjustments of blood-sugar are performed through the former mechanism.

Very extensive studies have been made on the influence which *muscular exercise* has on the amount of glycogen in the liver and muscles, but we are not aware of any modern research relative to the behaviour of the blood-sugar under such conditions. The general behaviour of the glycogen in the muscles has been fully reviewed by Pflüger (12), and we need do no more here than state the main conclusions that have been arrived at. In the first place, there is seldom more than 1 per cent. of glycogen in the muscles, the amount varying, however, in different animals. After death a glycogenolysis sets in, which can cause 88·4 per cent. of the original amount to disappear in four hours. Frequently, however, the glycogenolysis is much slower than this. Its occurrence is quite likely associated with the development of lactic acid, in connection with which it must be remembered that the presence or absence of oxygen is of great importance (see p. 165). Although, as we have seen, the muscles contain but a small amount of glycogenase, this enzyme is no doubt responsible for the glycogenolysis.

The influence of muscular exercise has been studied in various ways. The muscles of one leg (in frogs) have been tetanized, and the glycogen content compared with that on the other resting leg. The tetanized muscles were found to contain from 24·27 to 50·43 per cent. less glycogen than the resting.

In one of the hind-legs of the rabbit, after paralysis for several days as a result of nerve section, much more glycogen was found present than in the opposite active leg.

It is, of course, not alone the glycogen of the muscles, but also that in the liver and elsewhere in the body, that diminishes after muscular work. But it is very significant that the liver loses all of its store before the muscles lose theirs. The most important contributions in this connection are by Külz (22).

Glycogen furnishes fuel for the production of energy in muscular work, but it must become broken down into dextrose before it can be thus utilized, which accounts for the larger percentage of dextrose said to be present in muscle after tetanus than is present in resting muscle. A repetition of much of this work is, however, urgently required. To furnish fuel for this increased combustion, it would appear that the dextrose, mobilized in the liver and transported to the muscles by the blood, is more readily consumed than the dextrose which is produced from the glycogen of the muscles themselves, and this may be accounted for by the muscle glycogen being less hydrolyzable than that of the liver. The mechanism involved in this adjustment between the muscles and the liver has been discussed in another place ; it remains for us to find out with what degree of sensitiveness it acts. This could be ascertained by examining the percentage of sugar in the blood at different stages of muscular exercise, but, so far as I am aware, this has not been systematically done. It is usually considered that muscular exercise should be a part of the treatment prescribed in mild forms of diabetes. This is with the object of lowering the amount of available dextrose in the organism, and of stimulating its more active consumption. When this exercise is carried to exhaustion, however, it may cause the glycosuria in diabetic patients to increase. Such exercise is inadvisable in severe cases.

Increased combustion in the muscles may be produced in another way than by voluntary muscular exercise—namely, by rendering it necessary that more heat be produced because of increased

heat loss from the surface of the body. This increased combustion entails depletion of the glycogen stores in the liver; thus, if animals that have been injected with phlorhizin be given a cold bath and then kept in a cold room, their glycogen stores become much more quickly exhausted than is otherwise the case (23). This mobilization of glycogen has been said to be accompanied by changes in the concentration of sugar in the blood, a fall in external temperature causing, in dogs, a rise in the blood-sugar (24). Other investigators have, however, been unable to confirm these conclusions, either for dogs or rabbits (25). Thus in diabetic dogs (pancreatic and phlorhizin) a fall in external temperature does not cause an increase in the glycosuria (24A). It is interesting to note that the blood of small animals (guinea-pigs, cats) contains a higher percentage of sugar (dextrose) than that of larger ones (ox, horse) (26), and it is said that the blood of infants is similarly richer in sugar than that of adults (27). The explanation which is offered for these differences is that the relatively greater surface area in small as compared with large animals entails greater cooling, and a correspondingly more rapid demand for sugar on the part of the muscles. It is evident, however, that before such an explanation can be accepted, the effect of cooling itself must be definitely established.

We have been unable to make out any difference in the assimilation limit towards dextrose of small and large dogs, kept in cooled rooms (see p. 205).

It would profit us but little to take up each of the many conditions that may lead to the development of hyperglycæmia or glycosuria. In conclusion, however, it may be of value, for the guidance of further research, to indicate the general groups into which these conditions may be arranged. The following classification by Pollak (28) may be taken, for this purpose, as approximately correct:

1. Glycosurias dependent upon a change in the kidney:
   - (1) Without hyperglycæmia: Phlorhizin.
   - (2) With or without hyperglycæmia: Renal poisons (uranium salts).
2. Glycosurias directly dependent upon hyperglycæmia:
   - (1) Independent of the glycogen content of the organism: Pancreatic diabetes.

(2) Dependent upon the glycogen content (and acting through the nervous system).

(*a*) Central: Piqûre, caffein (diuretin), strychnine, afferent nerve stimulation, asphyxia.

(*b*) Peripheral: Adrenalin, asphyxia.

Regarding the nature of the change produced in the kidney by phlorhizin, we know little more than that it involves a complete alteration of permeability of this organ towards sugar, which therefore appears in large amounts in the urine, even after all the stores of glycogen have become exhausted, and the sugar concentration of the blood stands, if anything, below the normal.

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27. Frank and Cobliner : Zeitschr. f. physiol. Chem., 1910, vol. lx., p. 142.
28. Pollak : Archiv. f. exp. Path. u. Pharmac., 1909, vol. lxi., p. 376.

CHAPTER VIII

ASSIMILATION LIMIT OF SUGARS—GLYCONEOGENESIS

The animal body can assimilate large quantities of sugar. When, however, such quantities are overstepped, some of the sugar appears in the urine, and the amount of sugar which just suffices to cause this is known as its assimilation limit. There are at least three stages in its metabolism at which the sugar is delayed, and so prevented from overflowing into the urine. These are—(1) At the liver, where it is converted into glycogen; (2) in the tissues, where it is either converted into glycogen or oxidized; and (3) at the kidney, where it fails to be excreted unless its concentration in the blood has exceeded a certain degree. We have already seen that it is impossible to demonstrate any gradual increase in the reducing power of the urine when the amount of sugar is rising in the blood; it appears to remain stationary until a certain level has been overstepped. It is to be expected, therefore, that when quantities of sugar that are just short of the assimilation limit are ingested, no change will be detectable in the reducing power of the urine. At the same time, we must bear in mind that the tests upon which we have to depend for the detection of this glycosuria are quite arbitrary, and that there may be a preglycosuric stage during which the sugar content of the urine is rising. For the detection of this, however, more accurate methods of measuring the reducing power of the urine will have to be evolved.

When the assimilation limit for sugar is lower than usual, it indicates that something is amiss with carbohydrate metabolism. The fault may exist at any of the three barriers indicated above, and these we may narrow down to two by determining the amount of sugar in the blood. Such should be the practice in the clinical observation of diabetes. A knowledge of the assimilation limit is therefore an important aid in the investigation of

carbohydrate metabolism; for quite apart from the value to the physiologist of knowing just how much sugar the organism can assimilate, there is a great clinical importance attached to such knowledge. There can be no doubt that most forms of diabetes have a very insidious onset, and it is believed by the majority of clinicians that a diminution or total withdrawal of carbohydrates from the diet enables the organism to reacquire much of its lost power of assimilating these foodstuffs (*cf.* v. Noorden, Pavy, Lepine).

How important it must be, then, to test the carbohydrate-retaining power, for if it be found enfeebled, there is opportunity, by control of the diet, to improve it before it has become so upset that the organism begins to suffer from the toxic effect of excess of sugar.

Another point of practical importance concerns the expectation of life in persons with low assimilation limits for sugars. At present most insurance companies accept a negative test by Fehling's method as evidence of the absence of diabetes. They refuse all applicants whose urine shows reduction by this very approximate test. Is this not unfair both to the individual seeking insurance and to the companies ? For on the one hand, the glycosuria may indicate nothing more than the ingestion of a large excess of sugar with the food, and on the other hand, the urine of a really diabetic individual may have been " sugar-free " on account of dietary control.

Simple as the determination of the assimilation limits may seem to be, there are yet many sources of error, and unless certain precautions are taken, the results are quite apt to be inconstant and confusing. In brief, the observation is made by administering a weighed amount of the sugar in question, and thereafter examining the urine for the presence of sugar.

When the observations are to be made on laboratory animals, intravenous injection is probably the most accurate method to adopt for administration of the sugar, but in man the sugar must be given by mouth. In adopting this method, however, several sources of error are incurred on account of the various stages through which the sugar must pass during its digestion and absorption ; while in the gastro-intestinal tract it may, for example, suffer partial destruction on account of micro-organismal growth, so that the extent and nature of this may have a marked influence on the assimilation limit. Naturally,

too, the concentration of the sugar in the gastro-intestinal contents will affect the rate of its absorption, so that a certain amount of sugar will be absorbed at different rates, according to whether it is given alone or along with other foodstuffs.

Since it is with the utilization and control of the excess of sugar after it has gained entry to the blood that we are particularly interested (*i.e.*, with the glycogenic function and the subsequent utilization of the sugar), we must endeavour to eliminate as far as possible disturbing factors introduced by irregularity in the digestive and absorption processes. This, as already mentioned, can best be done by intravenous injection; but where we are compelled to substitute the method of oral administration, it becomes necessary to adopt particular precautions to maintain constant conditions in the digestive tract.

We shall first of all consider the results of *intravenous administration of the sugar*.

The most important researches of this nature are by Blumenthal and Pavy.

Both workers used unanæsthetized rabbits, the injections being made by means of a hypodermic needle inserted in the large ear vein. This operation does not in itself lead to glycosuria. In Blumenthal's observations (1) particular attention was paid, not only to the amount of sugar necessary, but also to the effect which the strength of the sugar solution and the rate of its injection might have on the occurrence of glycosuria. He found that two rabbits—the one weighing 2·6 kilogrammes and the other 3·2 kilogrammes—became glycosuric with 2·7 grammes dextrose; that less sugar had this effect when it was injected in dilute than in stronger solution; but that the rate of injection of the sugar solution—*i.e.*, from one to ten minutes—had no influence on the result. After an amount just short of the assimilation limit had been injected, a second injection within a short period of time immediately caused glycosuria, even when only small quantities of dextrose were injected, and this condition of saturation of the organism was sometimes maintained for hours. It may be concluded that the organism can very rapidly and efficiently remove any excess of sugar introduced into the circulation up to a certain point, beyond which, however, a certain portion of the excess passes into the urine. The above results show us merely how much sugar can be added at one time to the organism without any overflowing into the urine, but they furnish no information

regarding the power of the organism to utilize a constant though moderate excess of sugar. The one value Blumenthal calls the *saturation limit*, the other the *utilization limit*. This last is determined by injecting small doses at frequent intervals of time (every fifteen minutes for about an hour and a half). In three rabbits, each weighing about 2·8 kilogrammes, the utilization limit was found to vary from 0·033 to 0·06 gramme per minute ; in another smaller rabbit (1·9 kilogramme) it was 0·033 gramme.

Comparing different sugars in this way, it was found that the assimilation limits for dextrose and lævulose were about the same—viz., 2 to 2·7 grammes; then galactose, 0·4 to 0·6 gramme ; saccharose, 0·3 gramme ; and, least of all, lactose, 0·25 gramme.

It must be pointed out that the presence or absence of sugar in the urine in these experiments was ascertained by applying Trommer's test, comparison being made, when the results were doubtful, with normal urine. The objections to the use of this test have already been discussed. By the use of so arbitrary a test it is clear that there may have been a considerable excess of dextrose in the urine, as a result of the injection of amounts of sugar below the assimilation limit, without its being recognized.

From these observations we should have to conclude that there is no escape of excess of sugar into the urine until a certain level is reached in the blood. Such has been the commonly accepted view. Quite contrary to it, however, are the results obtained by Pavy (2) in a series of observations of the same general nature as those of Blumenthal, with the difference that, instead of merely using Trommer's test, the reducing power of the urine was determined by titration with ammoniated copper solution before and after boiling it with acid. In sixteen normal rabbits the average reducing power of the urine was found to be 0·369 per cent. before and 0·703 per cent. after hydrolysis.

When dextrose solution was injected intravenously at the rate of 25 to 30 c.c. per minute, and so that 1 gramme of dextrose per kilogramme body weight was administered, the urine collected within the next hour was found to contain, in an average of twelve rabbits, 15·6 per cent. more reducing substance than the normal. Even when so small an amount as 0·25 gramme was administered, the urine an hour later gave, in five rabbits, an average reducing power of 0·764 per cent. before, and 1·38 per cent. after hydrolysis.

The results of Pavy so far conform with those of Blumenthal

that about 1 gramme per kilogramme body weight of dextrose causes glycosuria. But, although at first sight there would appear, from Pavy's results, to be justification for the conclusion that only one-fourth this amount of dextrose also causes some increase in the dextrose content of the urine, more careful consideration of his figures does not bear this out. The following objections can be made to Pavy's results :

1. There are recorded only five experiments in which the urine was examined in sixty minutes after the injection. In one of these less than the normal amount of dextrose was found—viz., 0·228 per cent. before, and 0·567 per cent. after hydrolysis. In three, percentage amounts varying from 0·730 to 0·756 before hydrolysis and 1·332 to 1·768 after hydrolysis were found, which figures were, however, practically all below the *maximal* values given for the urine of normal rabbits—viz., 0·880 and 1·654. In only one of the five injected rabbits was any increase in reducing power observed in the urine before it was hydrolyzed.

2. For the estimation of such small amounts of reducing substance in urine a more accurate method than Pavy's must be employed.

3. In detecting the presence of mild forms of glycosuria, Pavy laid great stress on the value of a ratio which he called K, and which expresses the percentage of the initial reducing power of the urine to that obtained after hydrolysis. When this ratio is high, it indicates that a great part of the sugar is monosaccharide. In the five observations mentioned above, however, K averages 5·3 per cent., the average for the normal rabbit being 5·2 per cent. He explained this failure of K to behave according to the above conditions by supposing that the injected dextrose had become converted in the organism into some lower copper-oxide reducing state—*i.e.*, into maltose, glycogen, etc. This is, of course, a purely theoretical explanation.

Pavy submitted these results as evidence that the amounts of sugar in the blood and urine run parallel. It is evident that such a conclusion cannot be accepted.

With other sugars, Pavy obtained results which in general conform with those of Blumenthal. 0·25 gramme saccharose, per kilogramme body weight, caused distinct glycosuria, and by comparing the relative effects of 1 gramme per kilogramme on the reducing power of the urine it was found that lævulose and galactose caused, next to dextrose, the least increase, the disac-

charides, especially saccharose, causing a much more marked glycosuria.

In the case of the dog (unanæsthetized), about 0·4 gramme of dextrose has been found to cause glycosuria, although the more usual amount is from 0·8 to 0·11 gramme (3).

Although it is quite an easy matter to perform intravenous injection in the case of rabbits, this is a much more difficult operation in the dog. In place of it, several observers have employed *subcutaneous injection*. This means of administration of the sugar, however, involves a difficulty on account of uncertainty regarding the rate and extent of the absorption of the sugar into the blood. Even when there is only a small amount of fluid subcutaneously injected, and massage is practised to encourage its absorption, this may be quite tardy. In two hours after the injection, for example, a considerable amount of fluid may still remain at the seat of injection.

In rabbits Pavy found, when 1 gramme per kilogramme body weight was injected subcutaneously in 10 per cent. solution, that the urine which collected in the bladder within the following two hours showed very little increase in its normal reducing power for dextrose or lævulose—viz., 2·5 to 9·6 per cent.—or for maltose—viz., 1·3 to 10·7 per cent.—whereas with lactose and saccharose marked glycosuria occurred. Galactose had an effect intermediate between these two groups of sugars. The explanation offered for the similarity of the maltose with those of dextrose —a similarity which, as we have seen, does not exist when the intravenous method is employed—is that the maltose becomes hydrolyzed to dextrose by the action of maltase present in the tissues, so that it is absorbed into the blood as dextrose.

Underhill and Closson (4) found in female dogs that 3·5 grammes per kilogramme body weight of dextrose injected subcutaneously in 30 per cent. solution produced only the faintest glycosuria, and that this was even very slight in another dog receiving 7 grammes per kilogramme.

When the sugar is given *by mouth*, less constant results are obtained, unless special precautions are taken. This is shown in the Table on p. 203, which is taken largely from the work of de Filippi (6).

Although there is considerable discrepancy in many particulars, there are, nevertheless, certain important conclusions which can be drawn from the Table. The most important of these are as

ASSIMILATION LIMIT OF SUGARS IN NORMAL DOGS.

(Amounts are in grammes per kilogramme body weight.)

Author.	Dextrose.	Lævulose.	Saccharose.	Lactose.	Galactose.	Remarks.
Hoppe Seyler ..	—	—	20 to 30	—	—	Given along with a large amount of flesh.
Hofmeister (*loc. cit.*)	1·9 to 2·5 0·9 ,, 5·8	About same as dextrose	3·6*	0·4 to 0·8†	0·2 to 0·4	Sugars given along with meat. * Invert sugar. † Probably galactose.
Schlesinger ..	10 to 11	—	—	—	—	—
Luzzatto	—	—	—	1·1	0·6	—
Boeri and de Andreis	Starving, 4 to 6; well fed, 10 to 13	—	—	—	—	—
Quarta	Males, 4·06; females, 10·28	Males, 3.11; females, 3·58	—	Males, 1·54; females, 3·92	—	—
De Filippi	Males, 10	Males, 1·6	Males, 4	Males, 0·8	—	Dogs previously starved for eighteen hours.
Marine and Lenhart	Females, 8·7	—	—	—	—	Seven dogs starved eighteen hours; all had large goitres.

follows : (1) Lactose and galactose have much lower assimilation limits than dextrose, lævulose, and saccharose ; (2) male dogs can tolerate less dextrose than female, which is also the case, although to a less degree, with saccharose ; (3) less sugar can be tolerated in a starving dog than in one that is well fed. In connection with the last-mentioned point, it is interesting to note that starch itself can cause glycosuria in starved dogs, which is never the case in those that are well fed. Lactose is also better tolerated when it is given in milk than when it is given alone or with meat extract (Bovril), and excess of milk is more likely to cause glycosuria when it is given alone than when it is mixed with bread. According to Hofmeister (5), it is not on account of more rapid absorption that these results are due. However, it has been found that the administration of fat (lard) along with the sugar materially raises the limit (Cramer, 5A).

To insure reliable and constant results, we have found it necessary to observe the following precautions :

1. The dogs should be of the same sex throughout a given observation.

2. The diet should be of sufficient amount to cover the energy requirements and keep the dog in practically constant body weight. It may be a pure flesh or a mixed diet, but in the latter case the amount of carbohydrate given daily should be kept constant. During the actual experiment the animals should be kept in a room of equable temperature, and should not be unduly excited or frightened.

3. The sugar should be administered in water eighteen hours after the last meal. For doses of sugar amounting *in toto* to less than 50 grammes, a 10 per cent. solution should be employed ; for larger amounts, a 15 per cent. solution.

4. If, as in our own experience is usually the case, the dogs refuse to take the sugar, it should be given by the stomach-tube. It is well to make the solution very feebly acid with hydrochloric acid, and, while withdrawing the stomach-tube, to pour some water down it, so as to wash away any sugar solution from the œsophagus. By these precautions we have found vomiting to be much less frequent.

5. Every hour after giving the sugar the urine should be removed by catheter from the bladder, and examined for sugar. It is remarkable how quickly the dextrose appears in the urine when the assimilation limit has been exceeded.

When these precautions are taken, the individual differences in the assimilation limits of different dogs are of slight degree. For those that do occur, the age and the size of the animals would naturally be thought of as being responsible. So far as our experience goes, however, no constant difference can be made out on account of age. Regarding the influence of size, the greater relative surface area of small animals would lead us to expect that they could utilize more carbohydrate than large ones, especially when they are kept in a cool environment, for under such conditions it is known that the stores of glycogen in the body become quickly used up (see p. 193). On the other hand, it has been found that there is a considerably higher percentage of sugar in the blood of small, as compared with large animals (16), which must indicate, since the urine is sugar-free, that in those the kidney threshold for the escape of sugar into the urine is set at a higher level. This might lead us to expect a corresponding difference in the assimilation limits. As a matter of fact, however, it has been impossible so far to show that these are in any way different in the two groups of animals. Indeed, it is remarkable how closely the assimilation limits of a group of dogs of the same sex, and fed and housed alike, correspond. Thus, in seven female dogs observed by Marine and Lenhart (7), the variations were between 7 and 10 grammes (dextrose); in three females observed by Cushing, etc. (8), they were from 8·5 to 13·3, and in three males from 8·8 to 11·3 (cane-sugar).

Nor does the assimilation limit vary from time to time in the same animal—at least, over periods of several months. Occasionally, however, an animal is found with a very low limit. Thus, in one dog this stood at 3 grammes dextrose per kilogramme, and it was not affected by the animal becoming pregnant. And, again, it sometimes happens that the level gradually falls. Thus, Cramer (5A) observed a fall from 75 to 35 grammes dextrose in three months in the case of a three-year-old bitch.

Turning now to the observations on man, a few of the results are given in the Table on p. 206, largely from de Filippi (6).

Just as in the case of the dog, there is little uniformity in the results, and probably for the same reason: inconstancy in the condition of observation. Nevertheless, several interesting facts

ASSIMILATION LIMITS FOR SUGARS IN MAN.*

(Amounts are grammes of sugar just sufficient to produce glycosuria unless otherwise stated.)†

Observer.	Dextrose.	Lævulose.	Saccharose.	Lactose.	Remarks.
Worm-Müller	50 to 100	—	50*	100†	* Cane-sugar in urine. † Did not try less amounts of lactose on urine.
Moritz	200 +	—	—	—	—
Hofmeister	250 to 300	—	150 to 200	100‡	‡ Did not try less amounts, only dextrose in urine.
Zülzer	150	—	—	More than 100	—
De Rossi	—	140	—	—	—
Johannson, Billström, and Heijl	—	100	50	—	—
Brocard	—	—	Less than 75	—	—
v. Noorden	150 to 200	About 150	150 to 200	120 grammes	—

* Largely after Filippi.

† These are the amounts required for a man of average weight.

are clearly brought out. These refer to the results with lævulose, lactose, and saccharose.

Although the assimilation limit for lævulose appears to be not very different from that for dextrose, yet it is well to bear in mind that there is a considerable difference in the ease with which the organism metabolizes these two sugars, as is evidenced by the following facts: (1) The excretion of carbon dioxide is distinctly greater after ingestion of lævulose (and cane-sugar) than after an equal amount of dextrose (8); (2) the assimilation limit for lævulose, but not for dextrose, is said to be depressed when there is hepatic disease (9); (3) in diabetes, lævulose can sometimes be utilized better than dextrose (10).

Regarding lactose, it was found by Worm-Müller (11) to be this sugar which appeared in the urine when an amount over the assimilation limit was ingested. Under similar conditions in dogs, the excreted sugar was found to be not lactose, but probably galactose (5).

With regard to the chemical nature of the sugar which appears in the urine when an excess of *cane-sugar* is taken, the following observations are of interest (12): They were made on students varying in age between twenty and thirty years. The sugar was taken the first thing in the morning, followed by a small breakfast in about half an hour. The urine, before taking the sugar and during every ninety minutes afterwards, was collected and examined by the Nylander test, both before and after inversion. In all cases the urine, to start with, was sugar-free. In seventeen students, each of whom took 2·5 grammes of cane-sugar per kilogramme body weight, it was found, in one and a half hours, that the urine of ten contained cane-sugar, and that of three, invert-sugar; in three hours the cane-sugar had disappeared from the urine in two, but had appeared for the first time in another two (only two of the urines now contained dextrose); in four and a half hours, seven urines contained cane-sugar, but only one, dextrose. Even after six to seven hours, three of the above urines still contained cane-sugar. These results are clearly shown in the Table on p. 208.

In observations of older date by Stewart, in which excess of cane-sugar was taken by eleven students, this sugar appeared in the urine in eight, and dextrose as well in three.

In a similar experiment on twenty-nine students, the behaviour of the urine after taking 2·5 grammes of *dextrose* per kilogramme

body weight was studied. In one and a half hours, twenty-one showed glycosuria, which persisted for three hours in all cases. Of the eight that did not show it in one and a half hours, five became glycosuric in three hours, but there were three students who did not become glycosuric at all.

It may be a point of some diagnostic value in connection with incipient (so-called "latent") cases of diabetes to ascertain whether the ingestion of an excess of cane-sugar is followed by the appearance in the urine of dextrose or only of cane-sugar.

PRESENCE OF CANE-SUGAR AND OF DEXTROSE IN URINE AFTER INGESTION OF 2·5 GRAMMES CANE-SUGAR PER KILOGRAMME BODY-WEIGHT.

Subject.	1½ Hours.		3 Hours.		4½ Hours.		6-7 Hours.	
	C.S.	D.	C.S.	D.	C.S.	D.	C.S.	D.
S.	−	−	+	−				
D.	−	−	+	−	+	−	−	−
C.	+	−	+ ?	−	+	−	−	−
M.	−	−	−	−	−	−	−	−
G.	+	−	+	−	+	−	+	−
K.	−	−	−	−	−	−	−	−
B.	−	−	−	−	−	−	−	−
Jo. F.	+	+	+	+	+ ?	+		
G.		+		+		−		
Jo. L.	+	−	−	−	−	−		
P.	+	−	−	−	−	−		
R.	+	+	+	−	+ ?	−		
L.	+	−	+	−	+	−		
P.	−	−	−	−	−	−	−	−
G.	+	−	+	−	+	−	+	−
B.	+	−	+	−	+	−		
F.	+	−	+	−	−	−		

The amount of sugar which makes its appearance in the urine when quantities of sugars in excess of that which marks the assimilation limit are given, is only a very small fraction of the excess. The urinary sugar in such cases is not a mere overflow of the excess ingested ; on the contrary, even with enormous excess of sugar in the food, only a trace may pass into the urine. Thus in a healthy person v. Noorden (13) obtained the following results :

After taking—

100 grammes	saccharose,	0·00 grammes	appeared in urine.
150 ,,	,,	0·15 ,,	,,
200 ,,	,,	0·26 ,,	,,
250 ,,	,,	0·52 ,,	,,

And in a person observed by Worm-Müller (11), after taking—

50	grammes	saccharose,	0·10	gramme	appeared in urine.
100	,,	,,	0·85	,,	,,
250	,,	,,	1·80	,,	,,

When sugar is administered intravenously in considerable excess of the assimilation limit, an entirely different result is said to be obtained; there is, namely, an excretion of a great part of the excess by means of the urine. Thus Pavy injected two rabbits with 4 grammes of cane-sugar per kilo body weight and found the urine, excreted within less than two minutes after beginning the injection, to contain in one case 7·4 per cent., and in the other, 6·4 per cent. of cane-sugar. In similar experiments with dextrose, percentages of 3·9 and 4·7 of dextrose were found in the urine, indicating that much more dextrose had been retained in the organism than was the case with saccharose.

When the animals were not killed immediately after the injection, a greatly increased flow of urine was observed. Pavy remarks that "it is almost incomprehensible the rapidity with which the sugar in the circumstances reaches the urine." Approximate parallelism between the percentage of sugar in the blood and that in the urine also occurs when there is an excessive amount in the former as a result of increased sugar formation by the liver (see p. 47). In acute diabetes, however, this parallelism does not exist; there comes to be relatively *more* sugar in the urine.

It is clear from these observations that only a small fraction of the ingested sugar can find its way as such into the systemic blood; the bulk of it must be held back as glycogen in the liver. Excess of sugar could become added to the systemic blood, either because of the liver having failed to remove all of it from the portal blood, or because some has been carried into the systemic circulation by way of the thoracic duct. There is some evidence in favour of the latter view. Thus Schlesinger (14) found, after ligation of the thoracic duct in dogs, that about twice as much sugar could be tolerated as had previously been the case; and Schönborn (15) found the same to be true when the sugar was administered *per rectum* rather than when given by mouth, provided the injection was made above the level of the hæmorrhoidal veins.

The effect of hunger in lowering the assimilation limit can be more easily explained in this way than by supposing that the

liver has become depressed in its glycogenic powers. After starvation the absorption processes are probably very rapid, so that the lymph takes up more sugar than it ordinarily does, and adds it directly to the blood, where, as we shall see later, there is, despite the starvation, just as much sugar as usual.

When quantities of sugar that are less than the assimilation limit are ingested, there may be a fall instead of a rise in the sugar concentration of the systemic blood. It is difficult to harmonize this observation with the view that some of the sugar is absorbed by the thoracic duct unless we are to suppose that it is only when there is a high osmotic pressure of sugar in the intestinal contents that such occurs. The transitory nature of the glycosuria following excessive ingestion may be explained in terms of either view.

The assimilation limit in a healthy man for *polysaccharides*, at least for starch, is so high that when the starch is taken by mouth it is but rarely overstepped; for example, 6·4 kilogrammes dry ash-free starch was found to produce no glycosuria in man (Miura, 17). At the same time, all varieties of starch are not alike in this regard. Thus Schöndorff (18) found that many of the soldiers in the Bonn garrison were glycosuric, and that the sugar was derived from the bread that formed a large part of the daily ration. The beneficial action which oatmeal has, in certain cases of diabetes mellitus, seems to depend on something present in the grain rather than to a difference in the starch itself (19). Starvation materially lowers the tolerance for starch (20), but removal of the liver from the circulation, by the establishment of Eck's fistula (see p. 171), does not produce any alteration (21). When solutions of so-called soluble starch (which contains a mixture of amylose and dextrines) are injected intravenously, the starch quickly disappears from the blood, and does not pass into the urine unless the injections are rapidly made. No difference could be made out between injections made into the portal vein and those made into the jugular (20).

In the *diagnosis and treatment of diabetes in man*, especially in the milder varieties of the disease, the determination of the assimilation limit towards starch is of great importance. For this purpose, the patient, for some days after coming under observation, should be instructed to continue his usual diet, and the total daily sugar excretion—and that of nitrogen,

if possible—should be observed. The results give an approximate idea of the severity of the case. The diet should then be changed so as to consist of a part that contains no carbohydrate, and another, composed entirely of starchy food. The former is made up of eggs, meat, fish, green vegetables, fats, etc., and the latter should, to start with, consist of 100 grammes of bread distributed between the two main meals of the day, one of which should be breakfast. This diet should be continued with until the sugar excretion either disappears or attains a constant level. If it disappears, the case may be classified as a *mild one*, and the daily allowance of bread should be increased, by 50 grammes a day, until the sugar again makes its appearance in the urine, thus indicating that the assimilation limit has been reached. For therapeutic purposes, the patient should now be instructed to take about three-fourths of this amount of carbohydrate in his daily ration, and he should be supplied with explicit instructions—in the shape of diet tables—as to how he is to make this up ; that is to say, what varieties and quantities of carbohydrate foods he may take. His urine should be examined at frequent intervals—once a week—and he should be instructed as to the nature of his disease and the importance of his remaining aglycosuric. If possible, also, the percentage of blood-sugar should be determined from time to time. It is said, by those who have had great experience, that such cases may remain "latent" for many years, and that a gradual rise in the assimilation limit usually occurs.

When the glycosuria persists with 100 grammes of bread in the food, this must be reduced to 50 grammes a day, and, if this, after some days, does not suffice to render the urine free of sugar, carbohydrate must be withheld entirely from the diet. By thus reducing the carbohydrate gradually, danger of complications from increase of acetone bodies is avoided ; when, on the contrary, all carbohydrate is suddenly withdrawn, these may increase to a dangerous degree, and cause diabetic coma. Obviously the presence of acetone bodies in the urine should be observed during the treatment.

When the sugar disappears from the urine after the entire, or almost entire, withdrawal of carbohydrate from the food, the case is considered as one of *moderate severity*, and the treatment should be conducted along lines similar to those indicated above, with the additional instruction that on one day in every

week the diet should be composed almost entirely of green vegetables, with a moderate allowance of alcohol and plenty of fat.

If the case remains a-glycosuric, and the blood-sugar shows a constant tendency to decrease in amount, a small quantity of carbohydrate may be added to the diet, provided always that it does not cause any glycosuria. When even entire withdrawal of carbohydrate does not cause the sugar to disappear from the urine, the case is *a severe one*, and its treatment becomes much more difficult, for we have to watch not only the sugar excretion, but also that of the acetone bodies, and it becomes necessary to adapt the diet so as to avoid diabetic coma. It is in these cases that a certain amount of oatmeal has been found to have a beneficial influence. The treatment of such cases is, however, a problem of clinical medicine, and does not concern us here. In conclusion, it may be emphasized that, in trying the effect of a small increase in the carbohydrate allowance, the examination of the urine should extend over several days, instead of being confined, as it often is, to the day or so following the change. In very mild cases the assimilation limits of dextrose and cane-sugar should be determined, and the nature of the urinary sugar, when excess of cane-sugar is taken, identified (see p. 207).

Glyconeogenesis.

The formation of carbohydrate, especially of dextrose, out of molecules that do not belong to this group of chemical substances—for example, out of fats and proteins—occurs in an exaggerated degree in severe cases of diabetes, and possibly also to a certain extent in the normal animal. Apart from its purely biochemical interest, a knowledge of this process of glyconeogenesis, as it is called, is of great clinical value, for in the treatment of diabetes mellitus we must obviously know the sources of the sugar that is excreted in order that we may be able to reduce the amount of this substance in the body (1).

Although, on theoretical grounds, it may appear simple enough, by the so-called direct method, to determine what foodstuffs could yield carbohydrate in the animal body, this has not been the case in practice. In this direct method, as we have seen, we must show that the glycogen deposited in the liver—and the organism as a whole—is greater in amount than can be accounted for by the ready-made carbohydrate present in the body *plus*

that which may have been contained as such in the food. Even when the conditions are simplified by rendering the animal as nearly as possible free of glycogen before the foodstuff under investigation is given, the results are usually very uncertain. After all, however, this is to be expected when we bear in mind that the new sugar, instead of being stored as glycogen, may have been used up by the tissues as fast as it was formed. Quite apart from the difficulties that lie in the way of the investigation itself—*e.g.*, ridding the body of the glycogen, properly controlling the diet, etc.—the direct method cannot be expected to reveal glyconeogenesis in the case of foodstuffs from which produce only small amounts of carbohydrate are produced. For such the so-called indirect method must be employed. This consists in rendering an animal incapable of utilizing carbohydrate by making it diabetic, and then observing how the sugar excretion is affected either by feeding with a certain foodstuff, or by exciting the tissues to increased metabolism. In using the indirect method, however, it must always be borne in mind that the results obtained by means of it may be quite misleading when they are applied to the healthy organism. A normal animal, for example, may utilize protein and fat without converting any part of either into carbohydrate, whereas the power to do so may have developed in the diabetic animal as a result of the disease.

In the present chapter, therefore, we shall first of all consider glyconeogenesis in the diabetic animal, after which we shall proceed to see whether there is any evidence, derivable from a study of conditions that may be considered as intermediate between the pathological and the physiological, that the same process occurs in the healthy animal.

Three forms of diabetes have been used for the study of glyconeogenesis—diabetes mellitus, phlorhizin diabetes, and pancreatic diabetes. Although very important results have been obtained from clinical studies, we are compelled, from want of space, to confine our attention in this chapter mainly to those of the experimental observations.

The first step in such a research is to obtain evidence that the animal is completely diabetic. This cannot be done by merely estimating the amount of sugar in the urine. We must, besides, determine the nitrogen excretion, which will then serve as a standard with which that of the sugar may be compared. It has been usual to consider an animal as completely diabetic when

the ratio of dextrose to nitrogen (the so-called D : N ratio) is 2·8 to 1 or more. In accepting this standard, we are tacitly assuming that the dextrose is derived from protein, but that all the carbon of this is not so converted, for, if it were, 100 grammes of protein would produce 135 grammes of dextrose, and a D : N ratio of 1 to 8·45 would be obtained. Although this is, of course, impossible, it is nevertheless certain that more than 45 per cent. of the carbon, as represented in the ratio of 2·8 to 1, can go this way, for in phlorhizin diabetes, ratios of 1 to 3·2 are common, indicating that 60 per cent. of the protein carbon is appearing as sugar (2).

On account of this variability, the ratio for each animal must be determined as the first step in the investigation. In order to obtain one that is maximal and constant from day to day, however, certain experimental conditions must be fulfilled. In depancreated animals the extirpation must be very thorough (3), and in phlorhizin diabetes the animals must be injected every eight or ten hours with 2 grammes of the pure drug (Merck) dissolved in a weak solution of sodium bicarbonate.

The further investigation of the animal proceeds as follows : The urine of periods of twelve or twenty-four hours is collected, and the total amounts of nitrogen and dextrose estimated. For a day or so after the start of the observations the urine is usually excessively saccharine, because it contains the sugar of the glycogen previously stored in the body, as well as that derived from other sources. In order to accelerate the disappearance of the former, the animal should be given a cold bath, and be kept for some hours in a cold room. After it has been got rid of, a constant D : N ratio becomes established, and when it has been shown for several days that this is the case, the substance under investigation is fed to the animal. If this substance contains no nitrogen, and causes no change in the nitrogen excretion, any increase in that of dextrose must represent the extent to which it has been converted into this sugar. On the other hand, if the substance contains nitrogen, or if it causes a change in the excretion of nitrogen, it becomes necessary to calculate how much of the excreted dextrose might have been derived from the body protein (assuming that this can form dextrose), and how much from the administered substance. This calculation is made as follows : The amount of nitrogen in the administered substance is deducted from the nitrogen excreted, and the difference, which represents the nitrogen derived from the body protein,

is multiplied by the D : N ratio, which was obtained on the days previous to that on which the substance was fed. We obtain in this way the dextrose derived from the body. The dextrose derived from the administered substance can then be ascertained by deducting the dextrose derived from the body protein from the total dextrose excretion.

To serve as a check of the above method, especially in cases where the glyconeogenesis is slight in degree, another procedure can be adopted. This consists in feeding a diabetic animal for several days or weeks on carbohydrate-free food *plus* the substance under investigation, and then finding whether the total amount of dextrose excreted in this time, together with that present in the body at the end of the observation, exceeds that which could have been derived from the stores of carbohydrate originally present in the body. In using this method, we must make allowance for the *greatest* possible amount of carbohydrate that the body of the animal could contain under the conditions existing at the start of the experiment. Pflüger has insisted that this must be 4 grammes of dextrose per kilogramme of body weight, even in animals from which the pancreas has been for several days removed, or in those that have been for some time under phlorhizin.

Turning now to the outcome of the investigations, and first of all with regard to the evidence that glyconeogenesis does occur in the diabetic animal, we may take the following observations : a depancreated dog of 5 kilogrammes body weight excreted 1,176 grammes dextrose in twenty-five days, the food being free of carbohydrate. If we allow 4 grammes dextrose per kilogramme in the body to start with, 257 grammes might have come from this source, leaving 919 grammes which must have been newly formed (Lüthje, 4). In another observation of the same nature 2,499 grammes dextrose was formed in two months (Pflüger, 4A).

The possible sources of this dextrose are protein and fat. The evidence that it is derived—partly, at least—from protein is as follows :

1. The dextrose excretion in a diabetic animal rises and falls with the protein ingestion. Thus, a diabetic patient observed by Külz was fed for some time on carbohydrate-free diet, and then was given, on several successive days, measured quantities of casein along with meat extract and fat. The amount of dextrose excreted was found to run in the same direction

as the amount of casein in the food, as is shown in the following Table :

Food.	Sugar in Urine.
200 grammes casein	79 and 66 grammes.
240 ,, ...	70 ,, 66 ,,
300 ,, ...	87 ,, 97 ,,
500 ,, ...	137 ,, 127 ,,
240 ,, ...	87 grammes.

Although different investigators do not agree as to the exact order in which different proteins affect the dextrose excretion, yet there is no doubt that casein causes a much greater excretion than egg albumin ; that the proteins of blood and muscle occupy an intermediate position ; and that no difference can be made out between animal and vegetable proteins.

2. An increase in the sugar excretion occurs (in phlorhizin dogs) when they are given protein that has been completely digested with trypsin (Stiles and Lusk, 5), or when certain of the isolated products (amino-bodies) of such digestion are fed (6).

Since, in these cases, the amount of dextrose excreted bears a definite quantitative relationship to the amount of amino-body ingested, we are enabled to ascertain what proportion of the carbon of this substance has become converted into dextrose, and therefore we may draw conclusions regarding the nature of the chemical process by which the dextrose has been derived.

With regard to glyconeogenesis out of *fat*, it may be said that, so far, this has not been conclusively demonstrated, unless for the glycerine portion. To show it for fatty acid requires that the sugar excretion should be greater than can be accounted for on account of (1) the carbohydrate in the body, (2) the carbohydrate derivable from protein, and (3) the carbohydrate derivable from glycerine. Since we do not as yet know how much sugar might be derived, under the most favourable conditions, from protein (see p. 214), it is impossible to draw up a balance-sheet with sufficient accuracy. Giving fat to a diabetic does not produce an increase in the glycosuria.

Turning for a few moments to the nature of the chemical processes by which dextrose comes to be formed out of the products of fat (glycerine) and protein metabolism, the following facts have so far been established :

The simplest change is that which occurs in the case of *glycolaldehyde* ($CH_2OH—CHO$). In the laboratory three molecules of this can be caused to condense to form a hexose, and when it is

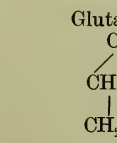
injected subcutaneously (in rabbits), a certain amount of dextrose appears within a short time in the urine. It is possible that glycolaldehyde might be formed in normal metabolism out of glycocoll (CH_2NH_2COOH).

Glycerine ($CH_2OH—CHOH—CH_2OH$) is also readily converted to hexose in the laboratory, becoming first of all oxidized to dioxyacetone ($CH_2OH—CO—CH_2OH$) and glyceric aldehyde ($CH_2OH—CHOH—CHO$). Two molecules of either of these may then polymerize to form a hexose. When this process occurs in the body, the hexose formed is dextrose. The sorbose-bacterium may also oxidize, and then polymerize glycerine to form a hexose.

Lactic acid ($CH_3CHOH—COOH$) is completely converted to dextrose in the body. This process must involve a rearrangement of the molecule, and subsequent polymerization (7). The related substance, propyl alcohol ($CH_3—CH_2—CH_2OH$), is also converted to dextrose in phlorhizin dogs.

Coming now to the amino-acids, it has been found that *glycocoll*, *alanin* (i), *aspartic*, and *glutamic acids*, cause a marked increase in the dextrose excretion of phlorhizin dogs, whereas *leucin* and *tyrosin* have no action in this regard. By determining the extent of the dextrose increase in the manner described above, Lusk and Ringer (2) have been able to show how the molecule of these substances must be split in order to produce the dextrose. The following Table is a summary of their results and conclusions :

(*Twenty grammes of the various amino-bodies were given to phlorhizin-diabetic dogs.*)

Acid and Formula.	Average Amount of Dextrose produced in Body.	Probable Change.	Dextrose that would be produced by Change.
Glycocoll CH_2NH_2COOH	13·43 (five dogs, one dog gave 15·77)	All C converted to dextrose	16·00
i. alanin CH_3CHNH_2COOH	18·77 (two dogs)	Do.	20·22
Aspartic acid $COOH—CH_2—CHNH_2—COOH$	12·42 (four dogs)	Three of the four C atoms converted to dextrose	13·52
Glutamic acid 	13·31	Three of the five C atoms converted to dextrose	12·24

These four amino-acids constitute about 36 per cent. of all the amino-bodies in flesh protein, being distributed as follows: Glycin 4, alanin 7·5, aspartic acid 9, glutamic acid 15·5 per cent. These quantities will yield a total of 26·3 grammes dextrose, thus accounting for about one-half of the 58 grammes which a diabetic animal produces from 100 grammes of flesh.

Leucin and tyrosin produced no change in the dextrose excretion. The essential difference between those amino-bodies which can form dextrose and those which cannot is further shown in their relative effect on the production of the so-called acetone bodies in the diabetic organism. Leucin and tyrosin cause the excretion of these substances to increase, whereas a decrease occurs when the dextrose-forming amino-bodies are given.

Although there is therefore no doubt that dextrose is readily formed from protein in the diabetic animal, we have yet to consider whether a similar transformation occurs in health. We will now very briefly review some of the experimental observations that are usually considered as evidence that it does.

After complete starvation for a long time—in the case of one dog for seventy-three days—considerable quantities of glycogen are quite commonly found in the body, and the blood-sugar, although it diminishes, never disappears. As we have seen, the most certain way of ridding the body of glycogen is by severe muscular exercise. After this has been terminated, however, the glycogen accumulates again. Such muscular exercise can be induced by strychnine. If the convulsions which are produced by this drug be abolished by giving chloral, and the animal kept alive for several days (without food), it will be found that glycogen has again accumulated in the body. In all those cases it must be from non-carbohydrate material that the dextrose (glycogen) is derived. The only interpretation which we can put on these facts is that life is impossible in the absence of the carbohydrate molecule, and that, when this is not available as such, it has to be manufactured out of other substances.

Evidence of an entirely different type is furnished by an examination of the respiratory quotient. It will be remembered that this expresses the proportion of oxygen absorbed by the animal to that of the carbon dioxide exhaled, and that it varies according to the nature of the foodstuff or tissue constituent that is undergoing metabolism at the time. When this is carbo-

hydrate, the quotient stands near 1, but when it is protein or fat, it falls to about 0·7. In starvation, however, quotients below 0·7 have been observed, which can be explained only by assuming that oxygen has been retained in the body beyond that which is necessary for immediate purposes of oxidation. Since it is known that this retained oxygen cannot exist in the body in a free state, we must conclude that it has been incorporated with certain substances, so as to raise their oxygen content. Such would be the case if proteins or fats, which contain only from 12 to 20 per cent. of oxygen, are converted to carbohydrates, which contain about 53 per cent. It does not necessarily indicate, however, that such a process occurs, for a similar retention would occur when oxy-acids, such as β-oxybutyric, are formed.

The undoubted conversion of at least protein and the glycerine portion of fat into carbohydrate in the diabetic and starved animal, and the possibility that fat may also be converted, raises the question as to whether, in the normal animal, proteins and fats may not become converted into carbohydrate before they can be utilized. Some have accepted this view, and have asserted that the process occurs in the liver. There are not wanting some observations that would seem to warrant such a conclusion. Thus, when an animal is first of all treated with strychnine, so as to render the liver free of glycogen, and this organ is then removed and perfused with defibrinated blood mixed with Locke's solution, it has been found that sugar accumulates in the blood up to a certain percentage, beyond which it ceases to be produced (7). But this can be shown not to be because of exhaustion of the glyconeogenic power of the liver, but because the sugar, which the perfusion fluid has gained, inhibits this process. The sugar formation, therefore, reappears when fresh blood-mixture is perfused. We must be cautious, however, in accepting results that are obtained on a perfused, and therefore half-dead, liver.

Assuming that the transference of oxygen necessary to convert proteins and fats to carbohydrate occurs in the liver, it is of interest to see what influence the removal of this viscus from the circulation will have on the respiratory quotient. It has been found, after clamping the aorta and vena cava so as to exclude the liver and the abdominal viscera from the circulation, that the respiratory quotient rises nearly to 1, and this has been interpreted as indicating that carbohydrates alone are being

burnt, and that no oxygen is being retained for the purpose of converting proteins and fats to carbohydrates (8). In passing, it should be pointed out that the respiratory quotient is very low in cases of diabetes, even when carbohydrate is being ingested, the interpretation which is usually given for this result being that no carbohydrate, but only fat and protein, is being oxidized. Indeed, this is usually considered as unassailable proof that the dextrose molecule cannot be oxidized by the tissues of the diabetic. It is plain, however, that the same result would be obtained if the liver had lost the power of transferring oxygen to the non-carbohydrate molecule as the first step in its utilization, and it is of very great interest, therefore, to note that the respiratory quotient of a depancreated dog has also been found to rise nearly to 1 when the liver is removed from the circulation. This would indicate that the diabetic organism can still use carbohydrate, but that the low respiratory quotient is due to inability of the liver to transfer oxygen to the fat and protein molecules.

By whatever chemical process they may become utilized, there is no doubt that fats and proteins supply the fuel upon which the diabetic animal lives, and it is probable that the proteins are much more available for this purpose than the fats. During the development of the diabetic state, as the animal is losing the ability to burn carbohydrates, it first of all falls back on protein, and when this no longer suffices to produce the necessary number of calories, fat may also become involved.

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